

# 5th Indo - Pacific Fish Conference

Nouméa - New Caledonia  
3 - 8 November 1997

## PROCEEDINGS

SÉRET B. & J.-Y. SIRE  
(editors)



Société Française  
d'Ichtyologie

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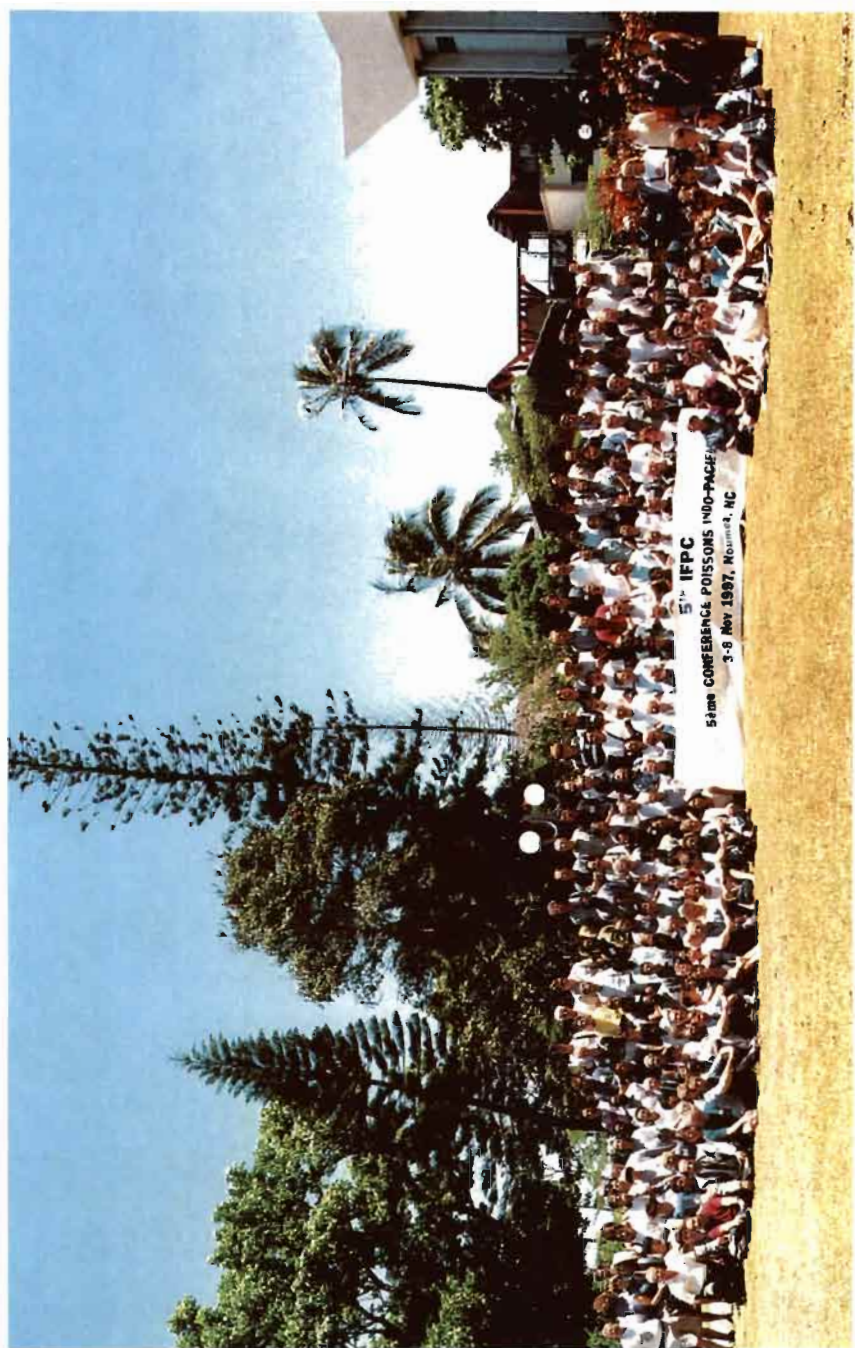


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## TABLE OF CONTENTS

Photography of the attendants	ii
Table of contents	iii-vii
Reproduction of stamps and coelacanth poster	viii
<b>Prefaces</b>	
Jean ALLARDI, Société Française d'Ichtyologie (SFI)	ix-x
Patrice CAYRÉ, Institut de Recherche pour le Développement (IRD, ex-ORSTOM)	xi-xii
<b>Allocutions</b>	
Dominique BUR, Délégué du Gouvernement en Nouvelle-Calédonie	xiii-xiv
Christian LÉVÊQUE, IRD	xv-xvii
Bernard SÉRET, SFI	xix-xx
List of referees	xxi
<b>Plenary lectures</b>	
BAUCHOT M.-L. - European exploration of the Indo-Pacific Ocean. [ <i>L'exploration européenne de l'Océan Indo-Pacifique.</i> ]	3-21
RANDALL J.E. - Zoogeography of coral reef fishes of the Indo-Pacific region. [ <i>Zoogéographie des poissons des récifs coralliens de la région indo-pacifique.</i> ]	23-26
<b>Symposium 1. Fresh and brackish water fishes</b>	
Chairperson: Tyson ROBERTS	
MARQUET G. & N. MARY. - Comments on some New Caledonian freshwater fishes of economical and biogeographical interest. [ <i>Remarques sur quelques poissons d'eau douce de Nouvelle-Calédonie présentant un intérêt économique ou biogéographique.</i> ]	29-39
MARQUET G., MARY N. & R. WATSON. - Comments on the freshwater fishfauna of French Polynesia. [ <i>Remarques sur l'inventaire des poissons d'eau douce de Polynésie.</i> ]	41-44
MARTIN-SMITH K., BULLOUGH L. & L. LAIRD. - Short-term movements of freshwater fishes in Sabah, Malaysia. [ <i>Mouvements à court terme de poissons d'eau douce à Sabah, Malaisie.</i> ]	45-56
JANEKITKARN S., PREMCHAREON S. & P. VITHEESAWAT. - Estuarine fishes of the Tha-Chin River, Samut Sakorn, Thailand. [ <i>Les poissons estuariens de la rivière Tha-Chin, Samut Sakorn, Thaïlande.</i> ]	57-64
SONTIRAT S. - Description of a new species of <i>Acantopsis</i> (Cypriniformes: Cobitidae) from Thailand. [ <i>Une nouvelle espèce d'Acantopsis (Cypriniformes: Cobitidae) de Thaïlande.</i> ]	65-68
MEISNER A.D. & B.B. COLLETTE. - Generic relationships of the internally-fertilized Southeast Asian halfbeaks (Hemiramphidae: Zenarchopterinae). [ <i>Relations phylétiques des demi-becs (Hemiramphidae: Zenarchopterinae) à fécondation interne du Sud-Est Asiatique.</i> ]	69-76
<b>Symposium 2. Coastal fishes of reefs, lagoons, and mangroves</b>	
Chairperson: Richard WINTERBOTTOM	
GILL A.C. - Subspecies, geographic forms and widespread Indo-Pacific coral-reef fish species: A call for change in taxonomic practice. [ <i>Catégorie taxinomique des poissons récifaux: espèces à large répartition géographique, sous-espèces ou formes géographiques? Invitation à un changement dans la pratique de la Taxinomie.</i> ]	79-87
GLUCKMANN I., BUSSERS J.-C., POULICEK M. & P. VANDEWALLE. - Preliminary study of the morphology of the head in Pomacentridae adductor mandibulae organization in <i>Dascyllus aruanus</i> (Teleostei: Perciformes). [ <i>Étude préliminaire de la morphologie de la tête des Pomacentridae: l'organisation de l'adductor mandibulae chez Dascyllus aruanus (Teleostei: Perciformes).</i> ]	89-97
HUTCHINS J.B. - Biogeography of the nearshore marine fish fauna of the Kimberley, Western Australia. [ <i>Biogéographie de la faune ichtyologique des côtes du Kimberley, Australie occidentale.</i> ]	99-108
MEUNIER F.J. & L. ZYLBERBERG. - The structure of the outer components of the scales of <i>Latimeria chalumnae</i> (Sarcopterygii: Actinistia: Coelacanthidae) revisited using scanning and transmission electron microscopy. [ <i>La structure des composants superficiels des écailles de Latimeria chalumnae (Sarcopterygii: Actinistia: Coelacanthidae) revue grâce au microscope électronique à balayage et à transmission.</i> ]	109-116
MEUNIER F.J. & H. FRANCILLON-VIEILLLOT. - Histological structure of the caudal spine of the surgeonfish <i>Ctenochaetus striatus</i> (Teleostei: Acanthuridae). [ <i>Structure histologique des épines caudales chez le poisson-chirurgien Ctenochaetus striatus (Teleostei: Acanthuridae).</i> ]	117-124
MEUNIER F.J., BÉAREZ P. & H. FRANCILLON-VIEILLLOT. - Some morphological and histological aspects of hyperostosis in the Eastern Pacific marine fish <i>Prionotus stephanophrys</i> Locking-	125-133

ton, 1880 (Triglidae). [ <i>Quelques caractères morphologiques et histologiques de l'hyperostose chez un poisson marin du Pacifique Est, Prionotus stephanophrys Lockington, 1880 (Triglidae).</i> ]	
PARMENTIER É., CHARDON M., POULICEK M., BUSSERS J.-C. & P. VANDEWALLE. - Morphological particularities of the head in four Carapidae (Ophidiiformes). [ <i>Particularités morphologiques de la tête chez quatre Carapidae (Ophidiiformes).</i> ]	135-146
<b>Symposium 3. Pelagic fishes</b>	
Chairperson: <b>Alexander HULLEY</b>	
COLLETTE B.B. - Mackerels, molecules, and morphology. [ <i>Maquereaux, molécules et morphologie.</i> ]	149-164
MCDOWALL R.M. & A.L. STEWART. - Further specimens of <i>Agrostichthys parkeri</i> (Teleostei: Regalecidae), with natural history notes. [ <i>Notes d'histoire naturelle sur quelques spécimens d'Agrostichthys parkeri (Teleostei: Regalecidae).</i> ]	165-174
<b>Symposium 4. Deep-sea fishes</b>	
Chairperson: <b>Tomio IWAMOTO</b>	
AKAZAKI M. & B. SÉRET. - <i>Dentex fourmanoiri</i> , a new species of sea bream (Sparidae: Denticinae) from off New Caledonia. [ <i>Dentex fourmanoiri, une espèce nouvelle de denté (Sparidae, Denticinae) de Nouvelle-Calédonie.</i> ]	177-183
DUHAMEL G. - Deep-sea demersal ichthyofauna off the St-Paul and Amsterdam Islands (central southern Indian Ocean). [ <i>L'ichtyofaune démersale profonde des îles St-Paul et Amsterdam (Océan Indien Sud).</i> ]	185-194
ROBERTS C.D. & T.C. GRANDE. - The sandfish, <i>Gonorynchus forsteri</i> (Gonorhynchidae), from bathyal depths off New Caledonia, with notes on New Zealand specimens. [ <i>Le "sandfish", Gonorynchus forsteri (Gonorhynchidae), des eaux profondes de Nouvelle-Calédonie et remarques sur des spécimens de Nouvelle-Zélande.</i> ]	195-205
<b>Symposium 5. Chondrichthyan fishes</b>	
Chairperson: <b>Bernard SÉRET</b>	
HOMMA K., MARUYAMA T., ITOH T., ISHIHARA H. & S. UCHIDA. - Biology of the manta ray, <i>Manta birostris</i> Walbaum, in the Indo-Pacific. [ <i>Biologie de la raie manta, Manta birostris Walbaum, dans l'Indo-Pacifique.</i> ]	209-216
BONFIL R. - Marine protected areas as a shark fisheries management tool. [ <i>Utilisation des zones marines protégées comme outil de gestion des pêcheries de requins.</i> ]	217-230
CARVALHO M.R. - A synopsis of the deep-sea genus <i>Benthobatis</i> Alcock, with a redescription of the type species <i>Benthobatis moresbyi</i> Alcock, 1898 (Chondrichthyes, Torpediniformes, Narcinidae). [ <i>Révision du genre Benthobatis Alcock, et nouvelle description de son espèce-type Benthobatis moresbyi Alcock, 1898 (Chondrichthyes, Torpediniformes, Narcinidae).</i> ]	231-255
FOWLER S.L., MANJAJI M.B., CAVANAGH R.D., COMPAGNO L.J.V., MYCOCK S.G. & P.R. LAST. - Elasmobranch biodiversity, conservation and management in Sabah (Malaysia). [ <i>Biodiversité, conservation et gestion des élamobranthes au Sabah (Malaisie).</i> ]	257-269
HAMLETT W.C., HYSSELL M.K., JEZIOR M., ROZYCKI T., BRUNETTE N. & K. TUMILTY. - Fundamental zonation in elasmobranch oviducal glands. [ <i>Zonation fondamentale des glandes nidementaires chez les élamobranthes.</i> ]	271-280
HAMLETT W.C., HYSSELL M.K., ROZYCKI T., BRUNETTE N., TUMILTY K., HENDERSON A. & J. DUNNE. - Sperm aggregation and spermatzeugmata formation in the male genital ducts in the clearnose skate, <i>Raja eglanteria</i> . [ <i>Agrégation du sperme et formation de spermatzeugmes dans les conduits génitaux mâles de la raie à nez blanc, Raja eglanteria.</i> ]	281-291
LAST P.R. & B. SÉRET. - Comparative biogeography of the chondrichthyan faunas of the tropical South-East Indian and South-West Pacific Oceans. [ <i>Biogéographie comparée des faunes chondrichthyennes tropicales de l'Océan Indien tropical du sud-est et de l'Océan Pacifique du sud-ouest.</i> ]	293-306
NAKAYA K. & K. SATO. - Species grouping within the genus <i>Apristurus</i> (Elasmobranchii: Scyliorhinidae). [ <i>Groupements d'espèces dans le genre Apristurus (Elasmobranchii, Scyliorhinidae).</i> ]	307-320
TANIUCHI T. & H. TACHIKAWA. - Geographical variation in age and growth of <i>Squalus mitsukurii</i> (Elasmobranchii: Squalidae) in the North Pacific. [ <i>Variations géographiques de l'âge et de la croissance de Squalus mitsukurii (Elasmobranchii: Squalidae) dans le Pacifique Nord.</i> ]	321-328
TESHIMA K., TODA M., KAMEI Y., UCHIDA S. & M. TAMAKI. - Reproductive mode of the tawny nurse shark, <i>Nebrius ferrugineus</i> (Elasmobranchii: Ginglymostomatidae) in Okinawa waters, with comments on individuals lacking the second dorsal fin. [ <i>Mode de reproduction du requin-nourrice fauve, Nebrius ferrugineus (Elasmobranchii: Ginglymostomatidae) dans les eaux d'Okinawa, et remarques sur des individus dépourvus de seconde nageoire dorsale.</i> ]	329-333
YANO K., YABUMOTO Y., TANAKA S., TSUKADA O. & M. FURUTA. - Capture of a mature female megamouth shark, <i>Megachasma pelagios</i> , from Mie, Japan. [ <i>Capture d'une femelle mature de requin grande gueule, Megachasma pelagios, au large de Mie, Japon.</i> ]	335-349
YANO K. - Chondrichthyans of the Ryukyu Islands, Japan. [ <i>Chondrichtyens des îles Ryukyu, Japon.</i> ]	351-365



## Symposium 7. Endangered species, consequences of species introduction

Chairperson: Christian LÉVÊQUE

- BRUCE B.D., GREEN M.A. & P.R. LAST. - Aspects of the biology of the endangered spotted handfish, *Brachionichthys hirsutus* (Lophiiformes: Brachionichthyidae) off Southern Australia [*Éléments de la biologie du poisson-crapaud tacheté, Brachionichthys hirsutus* (Lophiiformes: Brachionichthyidae), espèce en danger des côtes méridionales d'Australie.] 369-380
- CAPULI E. & R. FROESE. - Status of the freshwater fishes of the Philippines. [*Statut des poissons d'eau douce des Philippines.*] 381-384
- CASAL C.M.V., BARTLEY D.M., FROESE R., SA-A P. & M.L.D. PALOMARES. - Documenting the status of freshwater fish introductions in Oceania. [*Bilan des introductions de poissons d'eau douce en Océanie.*] 385-392
- GILL H.S., HAMBLETON S.J. & D.L. MORGAN. - Is the mosquitofish, *Gambusia holbrooki* (Poeciliidae), a major threat to the native freshwater fishes of South-western Australia? [*Gambusia holbrooki* (Poeciliidae) est-elle une menace pour les poissons indigènes des eaux douces du sud-ouest de l'Australie ?] 393-403

## Symposium 9. Phylogeography, genetic and chromosomal differentiation

Chairperson: Catherine OZOUF-COSTAZ

- ARNAUD S., BORSA P. & F. BONHOMME. - Mitochondrial DNA variation in the south-east Asian scad mackerel *Decapterus cf. macrosoma*. [*Variabilité de l'ADN mitochondrial chez le chinchard Decapterus cf. macrosoma* (Carangidae) en Asie du sud-est.] 407-411
- BURRIDGE C.P. - Suggestion of synonymy for *Nemadactylus* and *Acantholatris* (Perciformes: Cirrhitidae). [*Proposition pour la mise en synonymie de Nemadactylus et Acantholatris* (Perciformes: Cirrhitidae).] 413-416
- OOHARA I., SAWANO K., OKAZAKI T. & T. KOBAYASHI. - Reexamination of the molecular phylogeny of the masu salmon in the genus *Oncorhynchus*. [*Réévaluation de la position phylogénétique du saumon masou dans le genre Oncorhynchus.*] 417-426
- OZOUF-COSTAZ C., PISANO E., THAERON C. & J.-C. HUREAU. - Karyological survey of the notothenioid fish occurring in Adélie Land (Antarctica). [*Analyse caryotypique des espèces de Notothenioidei présentes en Terre Adélie.*] 427-440

## Symposium 10. Resource assessment

Chairperson: Guy DUHAMEL

- BERTRAND A., JOSSE E. & J. MASSÉ. - Preliminary results of acoustic target strength measurements of bigeye (*Thunnus obesus*) and yellowfin tuna (*Thunnus albacares*). [*Résultats préliminaires de mesures de la réponse acoustique individuelle chez le thon obèse (Thunnus obesus) et l'albacore (Thunnus albacares).*] 443-450
- DUHAMEL G., MAISON J. & P. PRUVOST. - By-catch in the deep-sea longline fishery of *Dissostichus eleginoides* (Nototheniidae) from the Indian sector of the Southern Ocean. [*Prises accessoires des pêcheries palangrières de fond de Dissostichus eleginoides* (Nototheniidae) dans le secteur indien de l'Océan Austral.] 451-458
- GRANDPERRIN R., AUZENDE J.-M., HENIN C., LAFOY Y., RICHER de FORGES B., SÉRET B., VAN de BEUQUE S. & S. VIRLY. - Swath-mapping and related deep-sea trawling in the southeastern part of the economic zone of New Caledonia. [*Cartographie et chalutages profonds dans la partie sud-orientale de la zone économique de Nouvelle-Calédonie.*] 459-468

## Symposium 11. Fish farming, aquarium fish issues

Chairperson: Roland BILLARD

- BILLARD R. - Biological diversity in pond fish culture. [*Diversité biologique en pisciculture d'étang.*] 471-479
- SAMANTARAY K., MOHANTY S.S., DASH G. & K. MISHRA. - The effects of acclimation temperature on the growth performance and the activities of aspartate and alanine aminotransferase of the air-breathing teleost, *Channa striata* (Channidae). [*Les effets de la température d'acclimation sur la croissance et l'activité de l'aspartate et de l'alanine aminotransférases chez le téléostéen à respiration aérienne, Channa striata* (Channidae).] 481-490

## Symposium 12. Reproduction, growth, physiology

Chairperson: François MEUNIER

- MARTIN-SMITH K. & L. LAIRD. - Reproductive patterns in some Cypriniformes from Borneo. [*Stratégies de reproduction chez quelques Cypriniformes de Bornéo.*] 493-504
- YAMAGUCHI A., HUANG S.-Y., CHEN C.-T. & T. TANIUCHI. - Age and growth of the star-spotted smooth-hound, *Mustelus manazo* (Chondrichthyes: Triakidae) in the waters of North-eastern Taiwan. [*Âge et croissance de l'émissole étoilée, Mustelus manazo* (Chondrichthyes: Triakidae), dans les eaux nord-orientales de Taïwan.] 505-513

Symposium 13. **Population and community ecology**Chairperson: **Yves LETOURNEUR**

- KOCHZIUS M. - Interrelation of ichthyofauna from a seagrass meadow and coral reef in the Philippines. [*Relations entre l'ichtyofaune d'un herbier de phanérogames et celle d'un récif corallien aux Philippines.*] 517-535
- SCHMITT R.J. & S.J. HOLBROOK. - Temporal patterns of settlement of three species of damselfish of the genus *Dascyllus* (Pomacentridae) in the coral reefs of French Polynesia. [*Chronologie de l'installation de trois espèces de poissons-demoiselles du genre Dascyllus (Pomacentridae) sur les récifs coralliens de Polynésie française.*] 537-551
- WOODLAND D.J. - An examination of the effect of ecological factors, especially competitive exclusion, on the distributions of species of an inshore, tropical, marine family of Indo-Pacific fishes (Siganidae). [*Analyse de l'influence des facteurs écologiques, notamment de l'exclusion par compétition, sur les distributions des espèces d'une famille de poissons tropicaux littoraux de l'Indo-Pacifique, les Siganidae.*] 553-562

Symposium 14. **Fish larvae**Chairperson: **Jeff LEIS**

- CASTLE P.H.J. & D.G. SMITH. - Precocious ovarian development in leptocephali of three Indo-West Pacific species of ilyophine eels (Teleostei: Synphobranchidae). [*Développement ovarien précoce chez les larves leptocéphales de trois espèces d'Ilyophinae (Teleostei: Synphobranchidae).*] 565-574
- LEIS J.M. & I.C. STOBUTZKI. - Swimming performance of late pelagic larvae of coral-reef fishes: *In situ* and laboratory-based measurements. [*Performances de nage des larves pélagiques âgées de poissons récifaux: mesures in situ et en laboratoire.*] 575-583
- NAKAZONO A. - A method to take photographs of live late larval and juvenile reef fishes. [*Une méthode pour photographier les stades larvaires avancés et les juvéniles de poissons récifaux vivants.*] 585-588
- RIEHL R. - Minireview. The micropyle of teleost fish eggs: Morphological and functional aspects. [*Brève revue. Le micropyle des oeufs des poissons téléostéens: aspects morphologiques et fonctionnels.*] 589-599

Workshop 1. **Parasitology and Ichthyology**Chairpersons: **Louis EUZET and Elisabeth FALIEUX**

- FALIEUX E. - What can parasite studies provide to Ichthyology? [*Qu'apporte l'étude des parasites à l'Ichtyologie ?*] 603-615
- NAHHAS F.M. & A. GREWAL. - Digenetic Trematodes from marine fishes of Fiji: subfamily Hurleytrematinae (family Monorchiidae): A review and description of four new species of Hurleytrematoides. [*Revue et description de quatre nouvelles espèces de Hurleytrematoides (Hurleytrematinae, Monorchiidae): Trématodes digènes parasites de poissons marins de Fidji.*] 617-631
- RIGBY M.C. - The Camallanidae (Nematoda) of Indo-Pacific fishes: Taxonomy, ecology and host-parasite coevolution. [*Les Camallanidae (Nematoda) des poissons indo-pacifiques: taxinomie, écologie et co-évolution hôte-parasite.*] 633-644

Workshop 2. **Databases on Indo-Pacific fishes**Chairperson: **Rainer FROESE**

- ALBERT J.S., FROESE R., BAUCHOT R. & H. ITO. - Diversity of brain size in fishes: Preliminary analysis of a database including 1174 species in 45 orders. [*Diversité de la taille de l'encéphale chez les poissons: analyse préliminaire d'une base de données comprenant 1174 espèces appartenant à 45 ordres.*] 647-656
- LABROSSE P., LETOURNEUR Y., KULBICKI M. & F. MAGRON. - FISHEYE: A new database on the biology and ecology of lagoon and reef fishes of the South Pacific. Example of its use on the ecology of commercial herbivorous fishes. [*FISHEYE: une nouvelle base de données sur la biologie et l'écologie des poissons récifaux et lagunaires du Pacifique Sud. Exemple de son utilisation en écologie des poissons herbivores commerciaux.*] 657-672
- SHAO K.T., CHEN J.-P. & S.-C. WANG. - Biogeography and database of marine fishes in Taiwan waters. [*Biogéographie et base de données des poissons marins de Taiwan.*] 673-680

Workshop 3. **Future of ichthyological research in the tropical Indo-Pacific**Chairperson: **Kent E. CARPENTER**

- CARPENTER K.E. & J.R. PAXTON. - The future of systematic ichthyological research in the tropical Indo-Pacific. [*Le futur de la recherche en Ichtyologie systématique dans l'Indo-Pacifique tropical.*] 683-693

#### Workshop 4. Fish collection management

Chairperson: Jean-Claude HUREAU

- FROESE R., BAILLY N., CORONADO G., PRUVOST P., REYES Jr. R. & J.-C. HUREAU. - A new procedure to evaluate fish collection databases. [*Une nouvelle procédure d'évaluation des bases de données de collections de poissons.*] 697-705
- HUREAU J.-C. - Co-ordination and standardisation of the management rules for fish collections. [*Coordination et standardisation des règles de gestion pour les collections de poissons.*] 707-716

#### Workshop 5. Fish poisoning: What do we know?

Chairperson: Jordi MOLGÓ

- MOLGÓ J. - Fish Poisoning: What do we know? Introduction to the workshop. [*Empoisonnements dus aux poissons: que savons-nous ? Introduction à l'atelier.*] 719-720
- MOLGÓ J., BENOIT E., LEGRAND A.-M. & A.S. KREGER. - Bioactive agents involved in fish poisoning: An overview. [*Agents bioactifs responsables des empoisonnements dus aux poissons: une vue d'ensemble.*] 721-738
- LEWIS R.J., JONES A. & J.-P. VERNOUX. - HPLC/Mass spectrometry and HPLC/MS/MS methods for the detection of multiple ciguatoxins. [*Méthodes d'HPLC couplées à la spectrométrie de masse pour la détection de diverses ciguatoxines.*] 739-744
- BENOIT E., MATTEI C., LEGRAND A.-M. & J. MOLGÓ. - Ionic basis of the neurocellular actions of Pacific ciguatoxins implicated in ciguatera fish poisoning. [*Bases ioniques des actions neurocellulaires des ciguatoxines du Pacifique.*] 745-758
- PAUILLAC S., SASAKI M., NAAR J., INOUE M., BRANAA P., CRUCHET P., CHINAIN M. & A.-M. LEGRAND. - Immunochemical methods for ciguatoxins detection in Pacific herbivorous and carnivorous fish. [*Méthodes immunochimiques de détection des ciguatoxines dans les poissons herbivores et carnivores du Pacifique.*] 759-773
- POLI M.A., MUSSER S.M. & S. HALL. - Laboratory diagnostics of brevetoxin and ciguatoxin intoxications in humans: Case reports and sampling considerations. [*Analyses de laboratoire pour diagnostiquer les intoxications humaines par les brevétotoxines et ciguatoxines: exposés de cas et conditions des prélèvements.*] 775-781
- ANGIBAUD G. - Clinical recognition of ciguatera fish poisoning in New Caledonia. [*Signes cliniques de la ciguatera en Nouvelle-Calédonie.*] 783-786

#### Workshop 6. Advances in fish behavior studies in the Indo-Pacific

Chairperson: Ernst REESE

- DONALDSON T.J. - Assessing phylogeny, historical ecology, and the mating systems of hawkfishes (Cirrhitidae). [*Évaluation de la phylogénie, de l'histoire écologique et des systèmes de reproduction des Cirrhitidae.*] 789-796
- FITZSIMONS J.M. & R.T. NISHIMOTO. - Application of fish behavior to stream monitoring on tropical pacific islands. [*Utilisation du comportement des poissons pour la gestion des rivières des îles tropicales du Pacifique.*] 797-804
- HOLBROOK S.J. & R.J. SCHMITT. - *In situ* nocturnal observations of reef fishes using infrared video. [*Observations nocturnes in situ de poissons récifaux par vidéo infra-rouge.*] 805-812
- NISHIMOTO R.T. & J.M. FITZSIMONS. - Behavioral determinants of the instream distribution of native Hawaiian stream fishes. [*Facteurs comportementaux de la distribution des poissons indigènes des rivières des îles Hawaï.*] 813-818
- LOSEY G.S. & T.W. CRONIN. - The UV visual world of fishes. [*Le monde visuel UV des poissons.*] 819-826

#### Workshop 7. From field to laboratory: Elasmobranchs as contemporary biological models

Chairperson: William C. HAMLETT

- HAMLETT W.C. - From field to laboratory: Elasmobranchs as models in contemporary biology. [*Du terrain au laboratoire: les élasmobranchs comme modèles en biologie contemporaine.*] 829-840
- WALLER G.N.H. - Magnetic resonance imaging study of the holotype of *Centroscymnus macracanthus* Regan, 1906 (Squaliformes: Somniosidae). [*Étude de l'holotype de Centroscymnus macracanthus Regan, 1906 (Squaliformes, Somniosidae) en imagerie par résonnance magnétique.*] 841-845

#### Workshop 8. Relations between man and fish in South Pacific cultures

Chairperson: Marie-Claire BATAILLE-BENGUIGUI

- BATAILLE-BENGUIGUI M.-C. - Men/fish relationships in traditional societies, their effects on human behaviour. [*Les relations homme/poisson dans les sociétés traditionnelles; leurs effets sur le comportement humain.*] 849-860
- PALOMARES M.L.D. - On the biological information content of common names: A quantitative case study of Philippine fishes. [*Sur l'information biologique contenue dans les noms vernaculaires: une étude quantitative sur les poissons des Philippines.*] 861-866



Reproduction of a velum drawn by B. Duhem, for the French Ichthyological Society on the occasion of the 5th Indo-Pacific Fish Conference. The original is preserved in the library of the Muséum national d'histoire naturelle, Paris.

**NOUVELLE-CALEDONIE**



5<sup>ème</sup> Conférence  
Poissons Indo-Pacifique

Premier jour d'émission



First day envelope with stamp illustrating the smallest seahorse of the world, *Hippocampus bargibanti* Whitley, 1970, edited on the occasion of the 5th Indo-Pacific Conference.

## PRÉFACE DE JEAN ALLARDI,

### PRÉSIDENT DE LA SOCIÉTÉ FRANÇAISE D'ICHTHYOLOGIE

La Société Française d'Ichtyologie a été créée en 1976 après le deuxième congrès des ichthyologistes européens qui s'était tenu à Paris. Forte de 335 membres dont 165 étrangers de 45 pays différents, la SFI a pour but de promouvoir l'ichtyologie fondamentale et appliquée. Ses actions se traduisent par l'édition d'une revue scientifique consacrée aux travaux d'ichtyologie, *Cybium*; l'édition d'ouvrages, par exemple celui sur la morphologie fonctionnelle du tégument écailleux des poissons de V. Burdak, traduit du russe en 1986, ou celui sur les *Rivulus* de Jean Huber, en 1992, ou encore celui de J. Depêche & R. Billard, "Embryology in fish. A review" édité en 1994, et l'organisation de réunions annuelles à thèmes. Enfin, un site web (<http://www.mnhn.fr/sfi/>) a été installé sur le site du Muséum de Paris pour informer les membres de notre société, et les autres ... C'est donc dans la continuité de ses actions, que la SFI a eu l'honneur de parrainer la 5ème Conférence sur les Poissons de l'Indo-Pacifique, et plusieurs de ses membres ont participé activement à son organisation en animant plusieurs symposiums ou ateliers.

Au cours de ces dernières décennies, la pêche s'est industrialisée en développant des technologies sophistiquées qui ne laissent que peu de chance aux poissons pour échapper à une capture certaine. Les ressources renouvelables menacent de s'épuiser et la majorité des stocks sont actuellement surexploités. Parallèlement, le développement de l'aquaculture, qui tend de plus en plus à remplacer l'exploitation des populations naturelles, génère de nouvelles nuisances (pollution, destruction d'habitat sensible, etc.).

La Conférence de RIO de juin 1992 sur la biodiversité avait mis l'accent sur la nécessité de la conservation de notre patrimoine biologique, et l'année 1998, proclamée année internationale de l'océan par l'ONU, s'est concrétisée par la tenue d'une exposition universelle, au Portugal, sur le thème « L'océan, un patrimoine pour l'avenir ». Ces manifestations témoignent du souci croissant de protéger, conserver et gérer la mer et ses habitants, à la fois en tant que ressources et en tant que nécessité écologique. Chacun de nous est en face de ses responsabilités. Les politiques, les économistes, les juristes doivent intégrer cette dimension universelle de l'unicité de la biosphère. Dans notre domaine, notre société s'efforcera d'inciter les pouvoirs publics à prendre en compte l'élément « poisson » dans les projets de développement et de gestion du patrimoine et de l'environnement, et à obtenir de ces instances les moyens nécessaires à la continuité des recherches en ichtyologie et à la formation de jeunes ichthyologistes. Ainsi, dans le cadre d'une gestion durable des ressources marines, lacustres et fluviales, nous oeuvrerons pour que la part d'incertitude dans les connaissances, qui fonde le principe de précaution, diminue chaque jour d'avantage.

La 5ème Conférence sur les Poissons de l'Indo-Pacifique de Nouméa a été une étape dans cette longue marche. Les textes présentés dans ces actes sont un témoignage de l'état actuel des connaissances, mais également un appel à la poursuite et à la diversification des travaux d'Ichtyologie dans cette vaste région indo-pacifique.



## FOREWARD BY DR JEAN ALLARDI,

### PRESIDENT OF THE FRENCH ICHTHYOLOGICAL SOCIETY

The French Ichthyological Society (SFI) was founded in 1976 following the 2nd Congress of European Ichthyologists held in Paris. Today, the SFI numbers 335 members, including 165 foreigners from 45 countries. Its aim is to promote fundamental and applied ichthyology. Its actions include the editing and publication of a scientific journal dealing with ichthyological research, *Cybium*, and of books: Burdak on the functional morphology of scale tegument of fish translated from Russian in 1986; the review of *Rivulus* by Jean Huber in 1992; and J. Depêche & R. Billard Embryology in fish. A review published in 1994, for example. Also, the SFI organizes annual meetings on specialised topics, and an internet web site (<http://www.mnhn.fr/sfi/>) linked to the Paris Museum site, to inform our members and the others about the Society. In the course of these actions the SFI was honoured to sponsor the 5th Indo-Pacific Fish Conference. In the organization of the Conference, SFI members were actively involved as chairs of symposia or workshops.

In the last decades, the fisheries have become more and more industrialized; the development of sophisticated technologies now gives few fish the chance to escape being caught. These resources might become extinct as, nowadays, most of the stocks are over-exploited. At the same time, the development of aquaculture, which tends to make up for the exploitation of natural populations, creates new problems (pollution, destruction of habitat, etc.).

The Conference on Biodiversity held in June 1992 in Rio stressed the necessity of conserving our biological heritage. In 1998, proclaimed by UNO international year for the ocean, the Conference created a worldwide exhibit in Portugal on the theme «The ocean, a heritage for the future». These events give evidence of the growing concern for protecting, conserving and managing the sea and its inhabitants, both as resources and as an ecological necessity. Each of us has to face this responsibility. Politicians, economists and lawyers have to integrate the universality of the biosphere with their work. In our field, our Society will try hard to prompt the public authorities to take into consideration the «fish factor» in development projects and in the management of our heritage and environment; and also obtain from these authorities the necessary means to continue research in ichthyology, and to train young ichthyologists. Thus, in the interests of developing the sustainable management of marine and freshwater resources, we will strive to reduce the uncertainties in our knowledge which are the basis for adopting a cautious approach.

The 5th Indo-Pacific Fish Conference held in Noumea was a step in this long process. The papers published in these proceedings provide state-of-the-art knowledge in our field. They are also a call for ichthyological studies in the vast Indo-Pacific region to be developed and diversified.

## PRÉFACE DU DR. PATRICE CAYRÉ,

DIRECTEUR DE RECHERCHE, CHARGÉ DE MISSION AUPRÈS DU DIRECTEUR  
GÉNÉRAL DE L'IRD POUR LES PROGRAMMES "RESSOURCES VIVANTES"

La série des conférences sur les poissons de l'Indo-Pacifique, initialisée par l'Australie (Sydney, 1981), a pour but de présenter les derniers résultats des études sur la biodiversité des poissons marins et d'eau douce de la zone indo-pacifique. Dans ce domaine de recherches et dans cette région, la France est présente par l'implication de divers organismes de recherche et universités, et tout particulièrement par l'ORSTOM, devenu depuis le 5 novembre 1998, l'Institut de Recherche pour le Développement (IRD).

Après le Japon (Tokyo, 1985), la Nouvelle-Zélande (Wellington, 1989) et la Thaïlande (Bangkok, 1993), la France a souhaité accueillir la 5ème conférence sur les poissons de l'Indo-Pacifique et en a confié l'organisation à l'IRD. Elle s'est donc tenue à Nouméa du 3 au 8 novembre 1997.

La conférence de Nouméa a réuni près de 300 personnes, provenant de 33 pays et environ 250 communications (200 présentations orales et 50 posters) ont été présentées dans 14 symposiums et 8 ateliers. Cette 5ème conférence a volontairement voulu faire évoluer les thèmes de recherche abordés en diminuant l'importance relative des communications sur la systématique qui était le thème principal des conférences précédentes, et en accroissant sensiblement la place réservée aux communications portant sur la biologie, sur l'écologie et sur l'éthologie des poissons. Les poissons cartilagineux (requins, raies et chimères) furent l'objet de 26 communications présentées dans un symposium et dans un atelier. Cet effort accru de recherche reflète l'inquiétude croissante concernant certaines espèces exploitées (les requins notamment) de ce groupe longtemps négligé. Les ateliers ont permis d'ouvrir la conférence à d'autres disciplines scientifiques telles que la parasitologie et l'ethnologie, de faire le point sur des questions particulières (e.g., l'ichtyotoxicité) et des questions d'actualité (e.g., le développement des bases de connaissances sur les poissons). Enfin, une réflexion sur l'avenir des recherches en ichtyologie tropicale dans l'Indo-Pacifique a montré les zones et les groupes sur lesquels l'effort devrait porter dans les années à venir pour expliquer l'existence d'un «triangle d'or» de la biodiversité dans cette région du monde. La tendance est donc d'allier, dans une dynamique de recherche plus moderne, les activités indispensables d'inventaire et de description, à celles de compréhension des mécanismes qui gouvernent l'évolution de cette biodiversité.

L'IRD remercie tous les participants qui ont contribué au succès de cette conférence et souhaite que les perspectives identifiées à Nouméa puissent trouver leur concrétisation au cours des prochaines conférences et notamment de la 6ème IPFC qui aura lieu à Durban (Afrique-du-Sud) en 2001.

## FOREWARD BY DR PATRICE CAYRÉ,

SENIOR SCIENTIST, CHARGÉ DE MISSION OF THE DIRECTOR-GENERAL  
OF THE IRD FOR THE "LIVING RESOURCES" PROGRAMMES

The series of conferences on Indo-Pacific fish, initiated by Australia (Sydney, 1981), is aimed at presenting the latest results of studies on the biodiversity of sea and freshwater fish of the Indo-Pacific area. France contributes to this field of research in the Indo-Pacific Region through the involvement of various research organizations and universities, and most particularly through ORSTOM, which has become, since 5 November 1998, the Research Institute for Development (IRD).

After the conferences in Japan (Tokyo, 1985), New Zealand (Wellington, 1989) and Thailand (Bangkok, 1993), France wanted to host the Fifth Indo-Pacific Fish Conference and entrusted IRD with its organization. This conference was convened in Noumea from 3 to 8 November 1997.

The Noumea conference gathered together almost 300 participants from 33 countries and about 250 presentations (200 oral and 50 posters) were made in 14 symposia and 8 workshops. This fifth conference aimed at an evolution of the research themes examined, by reducing the relative importance of the communications on systematics - which had been the main topic of the preceding conferences - and by increasing markedly the places reserved for presentations on biology, ecology and the ethology of fish. The cartilaginous fish (sharks, skates and chimaeras) were the subject of some 26 presentations in one symposium and one workshop. This increased research effort shows the growing anxiety concerning certain exploited species (notably sharks) of this long-neglected group. The workshops opened up the conference to other scientific disciplines, such as parasitology and ethnology, and provided state-of-the-art knowledge in response to particular questions (e.g. ichthyotoxicity) and on topical issues (e.g. the development of data bases on fishes). Finally, projections were made on the future of research in tropical ichthyology in the Indo-Pacific to determine the areas and groups on which efforts should be focused in the coming years in order to explain the existence of a « golden triangle » in biodiversity in this region of the world. The trend is therefore to combine, in a more modern research dynamic, the indispensable activities related to fauna inventories and descriptions with those linked to the comprehension of the mechanisms governing the evolution of this biodiversity.

The IRD thanks all the participants who contributed to the success of this conference and expresses the wish that the perspectives identified at Noumea will materialise in the course of the forthcoming conferences, in particular, at the 6th IPFC which is to take place in Durban (South Africa) in 2001.

## ALLOCUTION DE MONSIEUR D. BUR,

### DÉLÉGUÉ DU GOUVERNEMENT EN NOUVELLE-CALÉDONIE

Monsieur le Directeur,  
Monsieur le Représentant de sa Majesté l'Empereur du Japon,  
Mesdames et Messieurs les Consuls,  
Messieurs les Parlementaires,  
Messieurs les Conseillers,  
Monsieur le Représentant de la Société Française d'Ichtyologie,  
Amis de la Communauté Scientifique Internationale,  
Mesdames et Messieurs,

C'est pour moi un plaisir de souhaiter la bienvenue au nom du Gouvernement Français à une assistance aussi fournie et aussi éclairée.

Après Sydney, Tokyo, Wellington et Bangkok, Nouméa a la chance d'accueillir votre conférence qui rassemble plus de 250 participants extérieurs représentant 32 nations, dont la plupart des états riverains du bassin Pacifique.

Vos travaux sont traditionnellement orientés vers la classification des poissons de l'Indo-Pacifique, mais j'ai noté avec intérêt que d'autres aspects, à mon sens tout aussi importants, seront abordés durant cette semaine, à savoir la biologie, l'écologie et ses applications en matière de gestion ou d'exploitation de la ressource.

Il est en effet capital que des passerelles s'établissent entre ces diverses disciplines et que l'on débouche sur une vision plus systémique de la biodiversité. Cette conférence devrait vous en offrir l'occasion.

La conférence suivante ("Benthic Habitat"), organisée par la CPS, la SOPAC, l'ORSTOM et l'IFREMER est centrée sur les relations entre les ressources vivantes et l'habitat benthique représente une suite logique à cette interaction.

Depuis les quelques espèces de la zone antarctique jusqu'aux zones coralliennes de la mer de Corail ou des Célèbes, où la biodiversité marine atteint les plus fortes valeurs enregistrées, l'Indo-Pacifique représente un cas unique.

Cependant la très forte poussée démographique que connaît toute cette partie du monde engendre une exploitation de plus en plus sévère. Si on ne note pour l'instant que l'extinction d'une ou deux espèces, le marché du poisson vivant en Asie, le chalutage intensif et les filets dérivants géants sont des méthodes dictées par la recherche de la rentabilité immédiate.

L'essentiel de l'effort de pêche au niveau mondial relevant encore d'un comportement de type "cueillette", il est à craindre que les acteurs soient ramenés brutalement à la réalité par la simple dynamique des stocks et en la matière vos travaux, notamment sur le sujet très complexe du comportement des poissons, seront, je l'espère, de nature à mieux appréhender la gestion de cette ressource.

Dans ce vaste panorama, la Nouvelle-Calédonie occupe une position très particulière. Avec la Grande Barrière de Corail en Australie, elle détient une faune ichtyologique qui est l'une des plus diversifiées avec 2500 espèces identifiées pour un total probable de 3000.

Sur ce sujet, grâce à la présence de nos instituts tels que l'UFP, l'IFREMER ou l'ORSTOM, le niveau de connaissances a énormément progressé. D'abord limitées aux espèces lagunaires pour des raisons évidentes de proximité des activités humaines et de diversité, les études ont ensuite débordé sur la pente récifale externe, les monts sous-marins et le grand large.

Ces travaux ont donné lieu à d'importantes bases de données, la base de données "Fisheye" sur les poissons lagunaires et les deux bases de données "Pêche profonde" et "Grands pélagiques" du programme ZoNéCo qui vise à explorer les ressources marines de la zone économique de Nouvelle-Calédonie. Ces bases, dont certaines sont déjà accessibles par Internet, représentent pour les États de la région un élément incontournable dans la gestion future de la ressource qui, avec un chiffre d'affaires de plus d'un milliard de dollars pour les seuls thonidés, représente la première ressource du Pacifique insulaire.

On comprend aisément les raisons pour lesquelles les organisations régionales, et plus particulièrement l'ex-CPS devenue il y a quelques jours la Communauté du Pacifique, se sont focalisées sur cette question, avec en particulier la création d'une base de données régionale cette fois.

Avec une forte diversité, un effort de pêche moyen, voire faible ou carrément absent pour certaines zones et un bon niveau de connaissances, la Nouvelle-Calédonie représente un cas original.

Si son savoir commence à s'exporter, hier au royaume de Tonga en association avec la CPS, aujourd'hui aux Fidji, il n'en demeure pas moins vrai que le caractère de "laboratoire naturel" de certaines de nos eaux est de nature à attirer les chercheurs de la communauté scientifique. Sachez qu'ils seront les bienvenus et que la modélisation résultant d'une approche systémique sera probablement le seul outil de gestion dont nous disposerons avant la nécessaire transition vers l'aquaculture du troisième millénaire qui devra résoudre le fantastique défi posé par le déficit en protéines dans l'alimentation de l'humanité.

Je vous remercie de votre attention.



## ALLOCUTION DE MONSIEUR C. LÉVÊQUE,

REPRÉSENTANT DU DIRECTEUR GÉNÉRAL DE L'IRD

Monsieur le Délégué,  
Messieurs les Directeurs,  
Mesdames, Messieurs,  
Chers Collègues,

Les pêches, estimées à 100 millions de tonnes annuelles, contribuent en moyenne pour 20% à l'alimentation en protéines de l'humanité. Les économistes vous diront que cette ressource est un bien qui nous est fourni gratuitement par la nature, à l'encontre de la production contrôlée comme l'élevage et l'aquaculture, qui nécessitent des intrants parfois coûteux. Ce bien, la plupart d'entre nous reconnaitrons qu'il est nécessaire de le préserver. Sur les moyens d'y parvenir cependant, il n'y a pas toujours unanimité.

Le poisson est un élément clé des cultures asiatiques et du Pacifique. Alors qu'en moyenne la part du poisson est de 16 kg par personne et par an, elle atteint 28 kg dans l'ensemble Pacifique. C'est dire combien vous êtes concernés par la préservation et la valorisation de cette ressource.

Pendant longtemps, les recherches liées à l'exploitation des ressources aquatiques se sont limitées à la dynamique de la ressource. Des modèles sophistiqués de gestion de cette ressource ont été développés dans les années 1970. Beaucoup ont fait faillite car les prémices à l'origine de ces modèles ne prenaient pas en compte la variabilité de la ressource, non plus d'ailleurs que les stratégies des pêcheurs et le contexte économique d'une mondialisation des échanges. Ils ne prenaient pas en compte non plus l'évolution de l'environnement de cette ressource, que ce soit son environnement physique ou biologique. Or cet environnement se modifie, à la fois sous l'effet des changements globaux et des conséquences des activités humaines. Que sait-on des conséquences de la pêche sur la dynamique des écosystèmes qui peut, en retour, avoir des effets sur la ressource ? Que sait-on des conséquences de l'usage et de l'aménagement des milieux aquatiques sur la dynamique de la ressource ? Peu de choses en définitive au-delà de quelques anecdotes que colporte "l'écologie catastrophe".

Il s'est passé quelque chose en 1992, même si la mémoire de cet événement se perd un peu. La Convention sur la Biodiversité signée à Rio par la plupart des États n'est pas une convention très contraignante sur le plan juridique. Certains estiment même qu'elle est un échec relatif par rapport aux ambitions initiales.

Pourtant à la suite des discussions autour de cette convention internationale, la biodiversité est devenue un concept incontournable dans le champ de l'environnement, au même titre que l'effet de serre ou le développement durable.

Si l'on parle de biodiversité, c'est parce que la disparition accélérée des espèces vivantes et des écosystèmes dont elles dépendent est considérée comme une grave menace pour notre planète. La prise de conscience que les ressources naturelles ne sont pas illimitées n'est pas nouvelle, mais les moyens techniques dont nous disposons maintenant modifient de manière parfois irréparable la biosphère.

Le monde aquatique n'échappe pas à cette situation, bien au contraire. Les eaux continentales et côtières font l'objet d'une exploitation intensive, tout en étant le siège de pollutions diverses, de modifications des habitats, d'introduction d'espèces étrangères. Autant de choses dont nous discuterons au cours des prochains jours.

Dans la mouvance de la convention sur la biodiversité, la FAO essaie de promouvoir le principe de précaution au domaine des pêches. Le principe de précaution (l'approche précautionneuse), c'est en quelque sorte la reconnaissance de l'incertitude liée aux prises de décision en matière de gestion. Il donne une place importante au concept de développement durable en considérant que la gestion des pêches ne peut se faire indépendamment de la conservation de l'environnement. Il vise à promouvoir des pratiques de pêche compatibles avec les caractéristiques de l'écosystème.

L'approche précautionneuse prône donc une approche intégrée de la gestion des systèmes aquatiques. Le but ultime est de réduire les incertitudes en matière de décision. Mais, dans cette démarche, il s'agit surtout de ne pas négliger l'ensemble des stratégies d'acteurs. L'espoir réside dans le fait qu'une vision plus holistique de la dynamique des ressources vivantes permettra de mieux fonder les mesures de gestion appropriées. Celles-ci peuvent inclure à la fois la constitution des réserves, l'application de moratoires, une gestion flexible et adaptée à l'état des stocks.

Ne soyons pas naïfs. Il existe autour de l'exploitation des ressources aquatiques vivantes des enjeux économiques importants. La convention sur la biodiversité reconnaît les droits souverains des États sur leurs ressources biologiques. Elle recommande non seulement l'utilisation durable des éléments de la diversité biologique mais aussi le partage juste et équitable des avantages découlant de l'exploitation de ces ressources. Il est vraisemblable que la réticence de certains pays à signer la Convention tenait en partie à ces enjeux économiques. Pourtant, est-il incongru de penser que certains pays pourraient financer une partie de leur développement par une meilleure valorisation économique de ces ressources ? Nous rentrons ici dans un domaine hautement sensible qui touche aux conditions d'accès aux ressources et à leur exploitation. On a pu comparer l'exploitation des ressources de la biodiversité à l'exploitation pétrolière, avec des droits d'exploitation et des dividendes sur les prélèvements. Mais dans le domaine de l'ichtyologie, la question des brevets sur le vivant peut se poser également pour l'accès aux ressources génétiques.

Une idée qui prévaut actuellement dans les organisations internationales sur cette question de gestion des ressources est la part que peuvent jouer les connaissances traditionnelles dans le domaine de l'exploitation et de la gestion des ressources. Après une période au cours de laquelle on estimait qu'il n'y avait point de salut en dehors d'une gestion centralisée, voire étatisée des pêches, on s'est aperçu que cette approche aboutissait trop souvent à une absence réelle de gestion, l'État n'étant pas toujours capable de faire appliquer certaines décisions, d'ailleurs parfois discutables. On porte donc beaucoup d'intérêt à l'heure actuelle aux pratiques traditionnelles en espérant y trouver des voies nouvelles de gestion des ressources, même si l'on sait par ailleurs que ces pratiques ne peuvent plus être appliquées comme autrefois, compte tenu de la démographie et de la mondialisation de l'économie.

L'approche écosystémique prônée par la convention et par la FAO pose cependant quelques problèmes. Il s'agit d'intégrer les informations provenant de nombreuses disciplines, à des échelles de temps et d'espaces variables. Il faut intégrer en particulier les diverses influences anthropiques sur les écosystèmes.

Il faut développer les outils pour cela. Il faut innover par rapport aux modèles précédents, et rechercher des modes de représentation des connaissances mieux en rapport avec les conditions réelles.

Dans ce contexte, les ichtyologues ont leur rôle à jouer. Il s'agit en particulier de rechercher et de mobiliser l'information disponible sur les espèces, leurs exigences écologiques, l'impact des activités humaines. Il s'agit de développer des modèles prédictifs en termes de dynamique des populations dans un contexte environnemental évolutif, mais également, le plus souvent très variable.

L'enjeu est de taille, car il s'agit le plus souvent de dépasser le cadre de l'approche descriptive conjoncturelle et des anecdotes de l'écologie catastrophe, pour développer des principes et des modèles applicables à la gestion des ressources.

Néanmoins, nous savons également que les ichtyologues ne peuvent apporter qu'une compétence technique dans un contexte où les considérations socio-économiques priment trop souvent sur la gestion patrimoniale. Il y a eu, il y aura encore, beaucoup de déceptions. Le rôle du scientifique n'est pas de prendre les décisions, mais d'apporter aux techniciens et aux politiques les éléments qui leur permettront de décider en connaissance de cause. C'est par l'information, la sensibilisation des acteurs, la mobilisation de l'opinion que l'on pourra progresser. Il est donc important que les ichtyologues fassent connaître largement les résultats de leurs recherches. Le transfert des connaissances à tous les niveaux de la société est probablement la seule voie possible, même si elle peut paraître longue et parfois frustrante.

Quelques mots pour vous dire que l'institution que je représente ici, l'ORSTOM, s'est organisée pour répondre aux questions que je viens d'évoquer. Elle a recentré ses programmes autour de quelques grands axes correspondant à des préoccupations de développement. Deux d'entre eux nous concernent particulièrement ici :

- dynamique et usages des milieux aquatiques continentaux et des ressources en eau;
- dynamique et usage des écosystèmes marins et côtiers et de leurs ressources.

L'idée directrice dans la mise en place de ces axes de recherche est de mobiliser les compétences nécessaires pour une approche intégrée du fonctionnement de ces systèmes dans une perspective de gestion durable de leurs ressources. A partir des questions soulevées par les usages des milieux et des ressources, on essaie d'identifier les programmes de recherches nécessaires pour éclairer les décisions en matière de gestion. De tels programmes, le plus souvent multidisciplinaires, ne vont pas sans poser de problèmes de coordination qu'il faudra cependant surmonter pour répondre aux objectifs.

En conclusion, je dirai que le poisson est un excellent point d'entrée pour l'étude du fonctionnement des milieux aquatiques. Ressource économique, mais également modèle biologique par excellence, le poisson constitue une bannière autour de laquelle peuvent se regrouper tous ceux qui sont concernés par l'avenir des milieux aquatiques continentaux et marins. C'est en réalité le poisson, mais le poisson dans son milieu, qui doit constituer notre centre d'intérêt dans les années à venir. Il n'y a pas de salut pour les espèces aquatiques si l'on ne met pas l'accent sur la conservation des écosystèmes dans lesquels elles vivent.

Quelques orientations de nos activités en matière de conservation de la biodiversité sont possibles:

- promouvoir une approche intégrée des milieux aquatiques (approche précautionneuse);
- encourager le transfert et l'utilisation des connaissances dans une perspective de gestion durable;
- développer les outils multidisciplinaires de gestion d'une information comme les réseaux de bases de connaissances.

## ALLOCUTION DE MONSIEUR B. SÉRET,

VICE-PRÉSIDENT DE LA SOCIÉTÉ FRANÇAISE D'ICHTHYOLOGIE

Monsieur le Délégué,  
Messieurs les Directeurs,  
Mesdames, Messieurs,  
Chers Collègues,

Permettez-moi de vous adresser ces quelques mots au nom de la Société Française d'Ichtyologie.

La 5ème Conférence sur les Poissons de l'Indo-Pacifique a été placée sous l'égide de la Société Française d'Ichtyologie pour intégrer tous les organismes français effectuant des recherches dans les domaines variés de l'Ichtyologie. C'est ainsi que la plupart de ces organismes ont participé à l'organisation de cette conférence.

En premier lieu, il convient de remercier l'ORSTOM, principal maître d'oeuvre de cette conférence, mais aussi le Centre national de la recherche scientifique (CNRS), le Muséum national d'histoire naturelle (MNHN), l'École pratique des hautes études (EPHE), des universités et notamment l'Université française du Pacifique (UFP).

Le but de la Société Française d'Ichtyologie est de promouvoir tous les aspects de l'Ichtyologie. C'est ainsi que nous avons saisi l'opportunité de cette conférence pour renouer avec une tradition qui alliait l'Art et la Science à l'époque des grandes découvertes, en faisant réaliser une oeuvre d'art représentant un poisson.

Cette oeuvre d'art est un vélin, c'est-à-dire une aquarelle dessinée sur un support précieux: de la peau de veau mort-né. Le poisson sélectionné pour ce vélin est le "mythique" Coelacanthé, découvert en 1938 alors que l'on croyait le groupe éteint depuis le secondaire. Ce "fossile vivant", comme l'usage le qualifie, a donc traversé les ères géologiques, mais sa survie est fortement menacée du fait de la cupidité de certains hommes. Le vélin qui le représente a été déposé dans la collection du Muséum de Paris qui en compte près de 7000. Des reproductions seront diffusées au cours de la conférence.

A propos de grandes découvertes, je voudrais vous proposer un projet qui va vous paraître sans doute utopique, mais l'Histoire nous apprend que ce sont les utopies qui sont sources de progrès humain !

Dans moins de trois ans, nous changerons de millénaire ! A cette occasion, de nombreuses manifestations auront lieu dans le monde entier pour célébrer cet événement. Des sommes importantes vont être consacrées à des projets plus ou moins futiles.

Alors, dans le cadre de ce passage au troisième millénaire, pourquoi ne pas envisager un grand projet scientifique fédérant les pays de bonne volonté, pour organiser une grande campagne océanographique internationale, dans l'esprit du voyage autour du monde du "Challenger" à la fin du siècle dernier (1873-1876) qui marqua le début de l'océanographie moderne. Le but de ce projet, que l'on pourrait appeler "Challenger



2000", serait l'exploration des grandes profondeurs marines de l'Indo-Ouest-Pacifique qui sont encore largement méconnues.

En effet, nous envoyons des sondes spatiales pour savoir s'il y a de la vie sur la planète Mars, mais nous ne connaissons pas encore, ou à peine, nos "voisins" des abysses ! Et la "Mer de la Tranquillité" sur la Lune est sans doute mieux connue que la "Fosse des Mariannes" ! Pour ce projet, je pense particulièrement à la zone indo-ouest-pacifique, véritable "triangle d'or" de la biodiversité marine ! En effet, c'est la zone la plus riche de la "planète Mer" et c'est probablement la moins étudiée. Quel paradoxe ! Alors que depuis la conférence de Rio en 1992, la biodiversité figure souvent dans les discours politiques ! Voici l'occasion de concrétiser les bonnes résolutions prises à Rio. Au-delà de la connaissance scientifique "pure", un tel projet ambitieux serait susceptible de rapprocher les hommes, en mobilisant les énergies pour une oeuvre commune. Quand le premier homme a marché sur la Lune, nous avons la sensation d'être à sa place: c'était l'humanité dans toute sa diversité qui était présente sur la Lune avec l'astronaute Niels Amstrong !

Alors, ce projet "Challenger 2000" est-il une utopie ? À vous de le dire ...

# LIST OF THE REFEREES

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## **Plenary lectures**

- BAUCHOT M.-L. - European exploration of the Indo-Pacific Ocean. 3-21  
*L'exploration européenne de l'Océan Indo-Pacifique.*
- RANDALL J.E. - Zoogeography of coral reef fishes of the Indo-Pacific region. 23-26  
*Zoogéographie des poissons des récifs coralliens de la région indo-pacifique.*





## EUROPEAN EXPLORATION OF THE INDO-PACIFIC OCEAN

by

Marie-Louise BAUCHOT (1)

« The Grande Galerie of the Muséum national d'histoire naturelle in Paris has recently been renovated and opened to the public. Its exhibitions represent some of the accumulated scientific knowledge of the natural history of our planet. The displays are based mainly on specimens collected over the past 360 years, some of which have their origin in that vast body of water we know as the Indo-Pacific Ocean.

In contrast and juxtaposed to this monumental testimony to scientific endeavour, there is a small display outside the main entrance to the Grande Galerie. It contains a Micronesian navigational "chart", which shows the positions of the Marshall Islands (represented by small cowry shells) and the currents which relate to these islands (represented by an interlaced system of wooden strips). Such precise geographic and hydrographic details were known to the early Micronesian, Polynesian, Melanesian and Maori peoples. They mastered navigation by means of voyaging canoes and thanks to the changes in direction of the heavy swell on the open sea, as it meets with atolls, they were able to recognise a land even if it was out of sight, and determine their position in relation to the other islands.

Therefore at this, the 5th Indo-Pacific Fish Conference, I offer a challenge to all my colleagues, especially those from Pacific rim countries. I will take a totally Eurocentric stance in presenting my paper on the history of exploration of the Indo-Pacific by my Northern and Western Hemisphere antecedents. My challenge is that each one of you should examine your own histories of science and exploration, so that in the end we may be able to amalgamate these and eventually tell a story which will represent the many facets of a complex and often intriguing whole. Thereby it may be closer to the truth. I hope that my address will inspire you in your search. »

The middle of the XVIIIth Century used to be considered as the beginning of the great scientific maritime expeditions. At this time, sailors and scientists were convinced of the existence of a Southern Continent, but this *Terra Australia Incognita* - thought to be necessary to balance the mass of the continents of the Northern Hemisphere and thus ensure the stability of the globe - had not yet been discovered.

Before reviewing this era of great scientific expeditions, I should not ignore the explorers, who were curious to know what might exist beyond the known world. Thus I will briefly sketch the history of the discovery of the Indo-Pacific region.

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## XIVth AND XVth CENTURIES

In the XIVth Century, the "Great Ocean", which would later be called the Pacific, was a legend. If the populating stages in the Pacific ocean from South China and Java are now known (from 4,000 B.C. in New Guinea to 700-1,000 A.D. in New Zealand), modern historians are not sure whether European or Asiatic explorers actually reached it. According to some, Vikings had sailed as far as Polynesia, according to others the Chinese had explored the Mexican coasts before the XIIIth century.

The extent of the known world was considerably enlarged by two men during the XVth Century. Christopher **Columbus** discovered the New World in 1492, and since he sailed near the narrow strip of Panama missed the discovery of the Pacific Ocean by a hair's breadth. The Portuguese navigator **Vasco da Gama**, who by taking the eastern route via the Cape of Good Hope in 1497 (reached earlier by his compatriot **Diaz** in 1488), reached the East Indies and established the basis of an empire that would eventually extend as far as the Moluccas or Spice Islands.

In 1493 a series of papal edicts, especially the bull "Inter Caetera", put an end to the Hispano-Portuguese strife, because the Pope had drawn an imaginary line one hundred leagues west of the Cabo Verde Islands to divide the World into two parts: in the west the discovered land was to be Spanish; in the east Portuguese. In fact, the King of Portugal was displeased with this arrangement and, at the Treaty of Tordesillas (1494), had the line moved to 370 leagues west of the Cabo Verde Islands, so that the most eastern tip of Brazil became Portuguese.

## XVth CENTURY

At the beginning of the XVIth Century, the islands in the southern Indian Ocean, Madagascar (1500), Mauritius (1503) and Réunion (1528) were sighted by the Portuguese, among whom were **Petro Alvares Cabral**, **Alfonso de Albuquerque**, **Tristao da Cunha**, **Francisco Almeida** and **Pedro de Mascarenhas**.

The Moluccas, the famous Spice Islands, remained coveted by all. **Fernando de Magalhaes**, known in French and English as **Magellan**, claimed that he could reach them via the western route and by way of a southern ocean recently discovered by the Spanish navigator **Vasco Nunez de Balboa**. The latter had been able to cross the Isthmus of Panama overland in 1513. Rebuffed by the King of Portugal, **Magellan** obtained the necessary authorization from **Charles the Fifth** to launch his expedition (Fig. 1). On 20 September 1519, he set sail with five ships. The cosmopolitan crew was the cause of a bloody mutiny. In November 1520 and in spite of trying conditions - bad food, illness, ruthless discipline and inaccurate, and therefore dangerous navigational methods - **Magellan** succeeded in crossing the strait that bears his name and entered the Pacific Ocean. He reached the Marianas in March 1521 and then the Philippines. However, in the course of a battle between the natives of two islands opposite Cebu, he was killed by a poisoned arrow. **Juan Sebastian El Cano** took matters in hand and reached the Moluccas. Then, in conditions even worse than those that had prevailed during the Pacific crossing, he discovered the St Paul and Amsterdam Islands. He passed the Cape of Good Hope in May 1522, and sailed via the Cabo Verde Islands back to Spain, where he was awarded the honours that were due to **Magellan**.

The Venetian, Antonio **Pigafetta**, the chronicler of the voyage, did some observations of the natural history. He mentioned: « les poissons qui volent » (Fig. 2) and added « nous en vîmes en une si grande quantité ensemble qu'il semblaît que ce fut une île en mer ».

**Magellan** was the originator of voyages of circumnavigation around the globe. This success sharpened Hispano-Portuguese rivalry for the conquest of the World and mastery of the sea routes to the spice producers. This led to numerous expeditions motivated by political, commercial and religious concerns. These expeditions resulted in the discovery of New Guinea (1526) by the Portuguese **Garcia de Loyasa** and **Jorge de Meenes**. In 1528, the Spanish explorer **Alvar de Saavedra** touched the northern tip of New Guinea and landed at Eniwetok in the Marshall archipelago. Later (1568), the Spaniard **Alvar de Mendana** discovered the Solomons (Fig. 3) - rediscovered only two centuries later. On a second voyage (1595), **Mendana** and his pilot, the Portuguese **Pedro Quiroz**, landed at the Marquesas and Santa Cruz, an island of the New Hebrides (Fig. 4). **Quiroz** sailed once more in 1606 and discovered the Tuamotu and the island of Spiritu Santo in the New Hebrides.

## XVIIth CENTURY

From the beginning of the XVIIth Century, the Dutch also launched numerous expeditions to source spices. Between 1598 and 1603, twelve expeditions were undertaken by the Dutch. The foundation of the Dutch East Indian Company (VOC) in 1602 threatened the supremacy of Spain and Portugal, who had shared the World. The search for a southern continent also gave new impetus to numerous sea voyages and explorations. In 1616, **Jacob Lemaire** and the brothers **Willem** and **Jan Schouten**, Dutch merchants wishing to challenge the monopoly of the VOC, rounded Cape Horn on 29 February, reached Tonga and Java, and drew an approximate outline map of the New Guinea coast. Between 1616 and 1629, several Dutch navigators, among whom were **Dirks Hatichs**, **Pieter Nuyts** and **de Witts**, reconnoitred several parts of a large island which they called New Holland. The Governor of the VOC, **Anthony van Dieman**, entrusted **Abel J. Tasman** with the mission of exploring scientifically the southern Pacific. In 1642, **Tasman** sailed close to south Australian coast, convinced it was an island. He did discover Tasmania (which he called **Van Dieman's Land** in honour of the Governor of Batavia) and New Zealand, as well as several Tonga and Viti islands (later called Fiji, their name in the Tonga language), before reaching Batavia via the north coast of New Guinea. Later (1664), **Tasman** would explore the Gulf of Carpentaria and the Arnheim region.

The Iberian monopoly, already mentioned, was also attacked by the English. **Francis Drake** was the first Englishman to sail around the World between 1577 and 1580, an achievement duplicated by **Thomas Cavendish** between 1586 and 1588. One century later, and from 1683 onwards, a privateer named **William Dampier** undertook several extensive voyages. In 1688 and 1699, he was the first Englishman to explore in detail the little-known regions of Australia, and in 1700 to pass through the straits between New Guinea and New Britain. This was the time when the story of a Scots sailor, who was abandoned by mutineers in 1702 with three of his mates on an island of the Juan Fernandez archipelago and who was the only survivor rescued by Dampier in 1708, caused deep emotion in England. **Daniel Defoe's** famous *Robinson Crusoe*, published in 1719, was inspired by this adventure.

## XVIIIth CENTURY

In spite of the efforts made during the two centuries following **Magellan**, the knowledge of the Indian and Pacific Oceans remained fragmentary and scattered. All the navigators were hampered by the technical inadequacies of their ships and instruments, deficiencies the next period would overcome step-by-step.

At this time, a Dutchman, Jacob **Roggeveen** set sail in search of the Southern Continent. Starting from Texel Island in Holland, he reconnoitered Easter Island on April 14, 1722 and reached, via Tuamotu and Bora Bora, Samoa and Batavia in the same year.

I must also mention the English captain George **Anson**, who became First Lord of Admiralty and was considered to be the "Father of the Navy". After war had been declared on Spain, he set sail in 1740 to capture the galleons bringing back gold and silver from the American Colonies. He returned in 1744, having sighted no new land. He brought back gold and silver plundered on the coast of Peru, but of the 1,955 sailors manning his six ships, 1,051 had died of scurvy. Perhaps because of this disastrous loss of life, the story of his voyage, **Anson's** journal, met with considerable success. It gave rise to research on the methods to fight scurvy and to improve technical and human conditions during sea voyages.

A new era then begins in this history of discoveries - that of scientific expeditions. From 1763 onwards, France and England became ardent rivals. Governments became more and more interested in scientific developments, and learned academies and societies were created. At that time, science knew no frontiers, with scholars and scientists corresponding across the length and breadth of Europe. In France, Georges Louis **Leclerc de Buffon's** encyclopaedia (33 volumes published from 1749 onwards) comprised an inventory of scientific and technical knowledge. In Sweden, Carl **Linnaeus** devised a rational classification of nature. Cabinets of natural history became the fashion. Charles **de Brosse**, President of the Parliament of Burgundy and **Buffon's** childhood friend, published in 1756 *L'Histoire de la Navigation aux Terres Australes*, which met with considerable success. It was translated into several languages. It was he who coined the word "Polynesia". Shipbuilding improved dramatically. The first school for engineers in shipbuilding opened in Paris in 1765. Naval officers took scientific courses, so that those who led the expeditions were both excellent sailors and learned astronomers and cartographers capable of drawing reliable maps. In order to solve the fundamental problem of longitude, two methods were evolved: reckoning by lunar distance; and chronometers. These were real scientific break-throughs. Care was also taken with the quality of food and basic hygiene. Finally, no sea voyage was imaginable without the participation of groups of specialists in particular disciplines (astronomy, cartography and all the various branches of natural history), together with painters and draughtsmen.

Gradually this ideal system became the norm, in France thanks to Louis Comte **de Bougainville** and in England mostly to Captain James **Cook**, who set an example to be followed by their successors (Fig. 5).

Still with a view to the discovery of the Southern Continent, let me mention John **Byron's** voyage (1764-1766) (Fig. 6). He took possession of the Falklands and beat the record for crossing the Pacific Ocean, but did not discover any new islands. Samuel **Wallis** aboard the *Dauphin* (1766-1768) and Philip **Carteret** aboard the *Swallow* (1766-1769), after passing the Magellan Straits, went their separate ways. **Wallis** reached Tahiti and discovered numerous islands in the Tuamotu and Wallis archipelagoes, while

**Carteret** discovered Pitcairn on 2 July 1767 and several New Britain and Admiralty islands. On his way home, his path crossed with that of **Bougainville**, who had also sailed round the World.

Louis Antoine de **Bougainville**, a brilliant officer of noble birth, was admitted at the early age of 27 years to membership of the Royal Society of London, on the strength of his *Treatise on Integral Calculus*. He served under Montcalm in Canada. As a secretary in the French Embassy in London in 1754, he had the opportunity to exchange ideas with **Anson**. He set sail aboard the frigate *La Boudeuse*, which was closely followed by the store ship *L'Etoile* headed by **Chesnard de la Giraudais**. The expedition has been carefully prepared with the support of the Secretary of State for the Navy, the Duc de **Praslin**, and the help of de **Brosse** and **Buffon**. **Bougainville** had enlisted as specialists the astronomer Pierre Antoine **Véron** and the botanist Philibert **Commerson**, who was assisted by Prince Charles Othon de **Nassau**. The latter was fleeing from his creditors. After calling at the Malouines where he had established a colony in 1763 and which was given back later to the English under protest, **Bougainville** sailed through the Magellan Straits at the end of 1767. He reached Tahiti, which had been discovered in 1606 by the Spaniard **Quiroz**. He left us an idyllic description of the island. As he did not know that the Englishman **Wallis** had been there a few months earlier in 1767, he took possession of it in the name of Louis XV. It was there that the natives immediately recognized the true sex of **Commerson's** faithful valet, Jeanne **Baré**. After Tahiti and in 1768, the ships reached Samoa and a few of the islands in the archipelago which **Cook** would call the New Hebrides: Espiritu Santo Islands, Louisade Archipelago, Solomons, New Ireland, Moluccas and Ile de France. They were back in Saint Malo on 16 March 1769.

**Commerson** landed on Ile de France in December 1768 to study its natural history. It must be said that owing to « a hot-headed and violent disposition extreme in all things », he found it hard to bear the promiscuity of life on board « this bawdy house where hate, rebellion, dishonesty, banditry, cruelty and all sorts of disorders was the norm » (according to his own words). Returning from a voyage to Madagascar with **Sonnerat**, **Poivre's** nephew, he died from pneumonia on Ile de France on 13 March 1773. His notes and more than 1,500 drawings done with **Jossigny's** help were sent to **Buffon**, who made use of them to describe some birds. **Lacepède** used them to describe the fishes. The Jardin du Roi received 34 boxes of plants collected by **Commerson** (5,000 species, 3,000 of them new to science) and numerous dried fishes (Fig. 7). These were later discovered by **Cuvier**, who together with **Valenciennes**, described them in their *Histoire Naturelle des Poissons*. They are in the collections of the Muséum national d'histoire naturelle in Paris to this day.

James **Cook** was an exceptional man of modest birth. He was a self-taught sailor, having studied mathematics, geography and astronomy on his own. He was chosen by the Admiralty to lead an expedition with a dual purpose:

- to observe the passage of Venus in front of the sun, an eclipse which according to the great astronomer Edmond **Halley's** calculations was to occur on 3 June, 1769. Tahiti was identified as one of the most favourable vantage points to enable calculation of the distance of the earth from the sun;

- to reconnoiter the austral regions.

At the outset, nothing suggested that during his three voyages **Cook** was to become the greatest explorer of the Pacific Ocean (Fig. 8).

On 26 August 1768, he set sail on the *Endeavour* with 97 crew, among whom were several civilians: an astronomer, a natural history draughtsman - Sidney **Parkinson**, and two naturalists - Daniel **Solander**, a pupil of **Linnaeus** and Joseph **Banks**, an extremely wealthy young aristocrat who had a passion for natural history. The latter travelled with a number of servants and two greyhounds. **Cook** returned in July 1771, the voyage having proved an unqualified success. After calling at Tahiti in April 1769 to witness the passage of Venus, **Cook's** first mission was fulfilled. On 6 October 1769, he arrived in sight of a land that he took to be the Southern Continent. It was in fact New Zealand and he reconnoitered the whole coastline. Then on his way home he explored the eastern coast of New Holland (Australia).

From this first journey he brought back valuable geographic observations on the Pacific and rich collections for the naturalists: dried plants and seeds; mineralogical specimens; zoological collections with countless insects; bird and mammal skins; more than 500 fishes which were in alcohol; and 1,300 drawings (Figs 9, 10, 11) by **Parkinson** (who had died at the beginning of 1771). Several fishes given to Marie Auguste **Broussonet** by **Banks** himself are still today kept in the Muséum national d'histoire naturelle (Paris).

**Cook** had only spent a year on land when he undertook a second expedition with the *Resolution* and the *Adventure*. **Banks** refused to reduce the size of his team, twice as many as on the first voyage, and did not accompany him. The naturalists were Johan Rheinold **Forster**, a German living in London who had just translated **Bougainville's** voyage, and his 18 year-old son Johan Georg **Forster**, a remarkable artist who left us 78 drawings of fishes. The former was most unpopular because of his bad temper.

The idea of a legendary Southern Continent, which had been promoted for two centuries, was difficult to abandon. Therefore on leaving England in July 1772, **Cook** steered due south towards the Antarctic Ocean. In January 1773, he crossed the Polar Circle at 67°S, but due to the ice barrier he turned and steered a course towards New Zealand, the Society Islands and finally Tonga Tabou. Then he returned to New Zealand and sailed as far south as 71°S. He reached Easter Island in March 1774, followed by the Marquesas, Tonga Tabou, the New Hebrides (which he mapped) and finally on 4 September 1774 reached New Caledonia. He called one of the islands Pine Island because of its 200 feet-high conifers. In fact these giant pine-like trees are araucarias. He returned once more to New Zealand and was back in England on 29 July 1775 having sailed by way of the Cape of Good Hope and St Helena. In 3 years and 18 days he had travelled some 70,000 nautical miles and had lost only four men. His botanical, zoological and ethnographic harvest was considerable.

**Cook** came back convinced that if the Southern Continent existed, it was not within the reach of man. However, he was bent on solving another problem, namely the discovery of a passage between the Atlantic and Pacific Oceans to the north of America. This was the aim of his third expedition, which was to last 4 years and 2 months, on board the *Resolution* and the *Discovery*. There were no professional naturalists this time, but the Ship's Surgeon William **Andersen** was entrusted with the observations on natural history. Following the Cape of Good Hope route, **Cook** reached the Crozet and Kerguelen Islands. He then sailed towards the north via Tasmania, New Zealand, Tonga Tabou and Tahiti. In January 1779, he discovered an archipelago that no European had sighted previously, the Sandwich Islands (Hawaii). Here he was welcomed as a god. From Hawaii and via Alaska and the Aleutian Islands, which had been discovered by a Russian expedition in 1770-1771, he steered towards the straits reconnoitered in 1728 by Vitus Jonassen **Behring**, a Dane in the service of the Tsar. **Andersen** died in the Behring Straits, worn out

by consumption. **Cook** returned to Hawaii, where because of his ignorance of native customs and taboos he made mistakes that were to cost him his life. A punitive expedition against the natives of the large island took a bad turn, and he was killed with several of his men. The two ships once more tried to cross the Behring Straits but were eventually forced to sail back to England by hugging the coasts of Asia.

This genius **Cook**, as excellent an ethnographer as he was a sailor, had destroyed the two myths which had misled scientists and navigators for so long: the existence of a Southern Continent overflowing with riches; and a navigable northern passage between the Atlantic and the Pacific. Most important of all, he was the first to draw a precise map of the Pacific, which would prove to be essential to the success of all future expeditions.

As regards the history of the collections brought back by **Cook**, I suggest that **Peter Whitehead's** book (1968) should be consulted. **Whitehead** has published 40 drawings (37 for the first time) of fishes made by the artists of the three expeditions and has written the history of the collections, the manuscripts and the 262 drawings of fishes, some of which are now housed in The Natural History Museum (London).

King Louis XVI was among those who were enthusiastic about **Cook's** voyages and he wished that France could emulate these achievements. Unfortunately after **Bougainville's** half successful expedition on the scientific point of view, another led by Jean François **de Surville** in 1769 to New Zealand had been totally catastrophic. Marc Joseph **Marion Dufresne** and Julien Marie **Crozet** in 1771 led an expedition and on 23 January 1772 landed on two islands which they called "Iles de la Caserne", and were later (1776) renamed Marion and Crozet by **Cook**. Their venture ended the next year when the Maori people of New Zealand slaughtered **Marion Dufresne** and sixteen of his colleagues. Finally, Yves Joseph **Kerguelen de Trémarec** (1771-1772) discovered a small island in the southern Indian Ocean on 12 February 1772 (Kerguelen Island). This was not the Southern Continent he believed it to be.

After the Treaty of Versailles in 1783, which recognized the independence of America, France decided to challenge Great Britain at sea by continuing with the voyages of discovery and by commercial exploitation of the known regions.

With these two objectives in mind, a mission was extremely well planned by Charles-Pierre **Claret de Fleurieu**, Inspecteur au Service des Cartes et Plans de la Marine. Leadership was entrusted to Jean François de Galaup, Comte de **La Pérouse** (Fig. 12), who left Brest on 1 August 1785 on board the frigate *La Boussole*, accompanied by *L'Astrolabe* under Paul **Fleuriot de Langle**. This expedition brought together a comprehensive team of scientists equipped with the necessary sophisticated instruments required to fulfill a considerable scientific programme. Political and commercial interests were not neglected.

They sailed (Fig. 13) via Cape Horn, Easter Island and the Sandwich Islands to reach the coast of Alaska. Here 21 sailors died when their boats were smashed by the breakers. They then sailed to California and crossed the Pacific Ocean on their way to Macao and the Philippines. They explored the China and Japan Seas, Sakhalin, the Kuriel Islands and the Kamtchatka, which had been reconnoitered by the Russian **Khmiteffskoi**. From there **Barthélémy de Lesseps** set out for Paris across Siberia to deliver the documents and journals of the expedition. The ships steered due south to reach Samoa, where **de Langle** and 12 men were killed by the natives on 11 September 1787, a tragedy all the more unfortunate since **La Pérouse**, as a worthy heir to the philosophy of the Enlightenment, out of respect for the freedom of the encountered peoples, always refused

to take possession, in the name of the King of France, of lands which had not been explored yet. They landed at Botany Bay in Australia, from which they sailed on 10 March 1788 in a northerly direction. They were never seen again. Forty years later, remains of their wreck were identified by **de Lesseps**, who was the only crew member still alive.

Owing to the disruption caused by the French Revolution, it was only in 1791 that Joseph Antoine Raymond, Chevalier de Bruny **d'Entrecasteaux** and Jean Michel **Huon de Kermadec** on board *La Recherche* and *L'Espérance*, were commissioned to look for **La Pérouse's** remains among the hundreds of islands in the south-eastern Pacific. They did a remarkable job, exploring numerous coastlines particularly those of New Holland, New Caledonia (1792), New Britain and New Guinea. During the voyage they discovered an island which did not appear on any map and called it Research Island (Fig. 13). It was in fact Vanikoro, where **La Pérouse** had been shipwrecked. But **d'Entrecasteaux** went further in search of **La Pérouse's** remains to the Solomon and Louisiade archipelagos. The two commanders responsible for the expedition and several sailors died at sea in 1793. The rest of the crew, consisting of Royalists and Republicans clashed violently. They were taken prisoner by the Dutch and returned to France in 1796. Some only returned in 1797. Even if the expedition did not solve the mystery of **La Pérouse's** disappearance, it brought back a great wealth of scientific documentation. Due to the hostility of Holland and Great Britain towards France, all of these had been transferred to Great Britain. However, thanks to Sir Joseph **Banks'** intervention, they were eventually restored to France and the collection entrusted to the Muséum national d'histoire naturelle in Paris. It should be noted that the British Admiralty used the opportunity to copy all the maps.

## XIXth CENTURY

Against the background of continuing Anglo-French rivalry, further expeditions were undertaken. The French did not want the English to be the only settlers in Australia, and the English were afraid that France might establish a colony on Van Dieman's Land. In France, Nicolas **Baudin's** voyage (1800-1804) was organized (Fig. 14), and in England, that of Matthew **Flinders** (1801-1803). Officially these missions were scientifically and not politically motivated.

**Baudin's** expedition on board the corvette *La Géographe* was remarkably well prepared by a commission presided over by First Consul **Bonaparte**, and included such eminent personalities as **Bougainville**, **Lacepède**, **Cuvier** and **Jussieu**. **Baudin** was accompanied by Emmanuel **Hamelin** on the store ship *Le Naturaliste*. Both ships were to suffer numerous mishaps. There were clashes between the authoritarian **Baudin** and several inept officers and the 24 scientists which the Institut and Muséum had foisted on the expedition. **Baudin** failed to comply with naval regulations and dozens abandoned ship at Ile de France. To make matters worse, there was much illness and many deaths during the 3 years and 5 months spent at sea. Thirty-two of the company died, including **Baudin**. But in spite of this, the scientists who took part in the venture were extremely successful in their mission. Who can ignore the names of the zoologist François **Péron** and the future artist and naturalist Charles **Lesueur**? The latter, who had signed on as a gunner's mate before his talent as a painter was recognized, and Nicolas **Petit**, who had studied academic art in **David's** studio, executed more than 1,500 drawings and paintings relating to natural history. **Péron** brought priceless collections back to the Muséum - according to **Jussieu** and **Cuvier** these included 100,000 specimens, amongst which were 2,500 undescribed species, including more than 200 fishes.





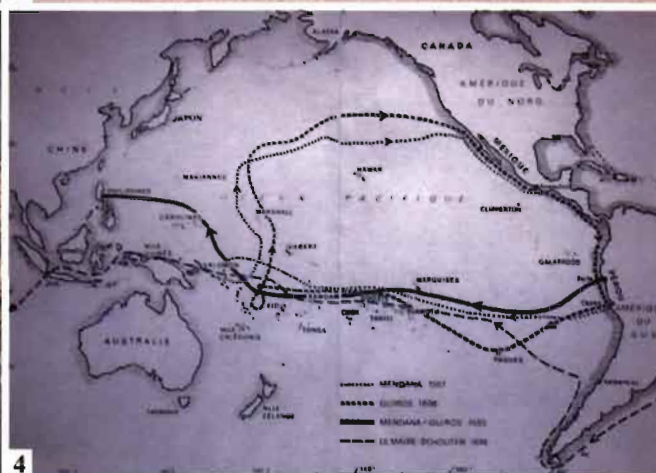
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Fig 1. - Magellan's expedition. Old map from "Atlas antique", Agnese, 1543. Map reprinted from Jacques Brosse "Les tours du Monde des explorateurs" © Bordas, Paris, 1983. Fig. 2. - Painting done after Pigafetta's description during Magellan's voyage; he reports on « fish that fly », « we saw such a great amount that it looks like an island ». Reprinted from Taillemite, © Gallimard, Paris 1995. Fig. 3. - Abraham Ortelius's map of the world, from the great Atlas of 1570. Reprinted from Taillemite, © Gallimard, Paris 1995.

Also in 1801, **Flinders** set sail aboard the *Investigator* to explore the greatest part of the New Holland coasts. He had received financial assistance from Sir Joseph **Banks**, who had personally chosen a group of naturalists. Amongst these were the botanist Robert **Brown** and the draughtsman **Brauer**. The hydrographic work was much more precise than **Baudin's**. However, this voyage became a succession of dramas, what with the loss of several sailors, damage to the ships when sailing in the Great Barrier Reef region, and **Flinders'** arbitrary confinement on Ile de France for 6 years. Nevertheless, **Brown** brought back a considerable herbarium, which enabled him to publish the first work of the New Holland flora.

### RUSSIAN EXPLORATIONS

The great Russian explorations must also be mentioned. **Peter the Great** and his successors wanted to end Russia's isolation and transform it into a modern power, possessing an army and a navy capable of playing a role in the world. In 1725 and in the year that he died, **Peter the Great** launched an expedition to try to establish whether Siberia and North America were separated by sea. In 1728, **Behring** mapped the straits named after him, and in 1740-1741 he explored Alaska and the Aleutian Islands. By 1739, Russian ships had reached the coasts of Japan. In 1743, **Khmitteffskoi** reconnoitered the coastal regions of Siberia as far as Kamchatka.

At the beginning of the XIXth Century **Alexander the First** wished to improve the fur trade with North-west America and to lead a Russian delegation to Japan for political objectives. He therefore organized a maritime expedition under **Krusenstern** (1803-1806) (Fig. 15). The Tsar took care in enlisting scientists such as Wilhelm Gottlieb **Tilesius von Tilenau** and Georg Heinrich Baron **von Langsdorff**, both of whom he recruited at German universities. **Von Langsdorff** left the expedition in Kamchatka and returned to St Petersburg on foot and by horse. The *Nadjedjeda* and the *Neva*, two strong ships built in England, sailed from Cronstadt in August 1803 and returned 3 years and 12 days later without having lost a single man. This proved that the Russians could compete with their powerful British and French rivals. The naturalists brought back a rich harvest of valuable information on the flora and fauna of the countries that they had visited. Prior to an un-edited contribution by **Tilesius**, little was known about life in the oceans. Thanks to him, several species of Gadidae and Blenniidae from the Sea of Okhotsk were described. **Krusenstern** published an excellent atlas of the Pacific following another voyage of exploration to the Behring Straits in 1815.

Subsequent Russian expeditions bring to mind the one made by Otto Levstafievitch **Kotzebue** aboard the *Rurik* (1815-1818). On board were the naturalist and poet **Chamisso** and the painter Louis **Choris**, who illustrated many of the fishes during the exploration of the East Caroline Islands. We remember especially Fedor Petrovitch **Lutke's** expedition on the *Seniavine* (1826-1829) and his careful exploration of the Caroline Archipelago. On board were Alexander **Postel**, artist and mineralogist, the ornithologist Baron **Kittlitz** and the naturalist Karl Heinrich **Mertens**. They examined numerous fishes and painted 1,300 remarkably accurate water-colours of which 254 painted by **Postel** represented the 300 fish species that were caught. The drawings were identified by **Cuvier** during a visit that **Kittlitz** and **Postel** paid to him in Paris.

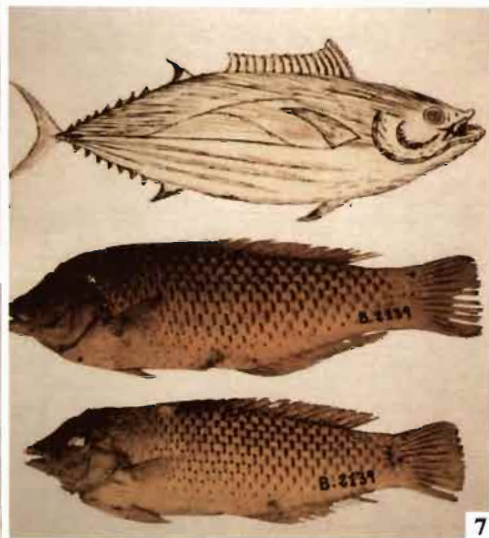
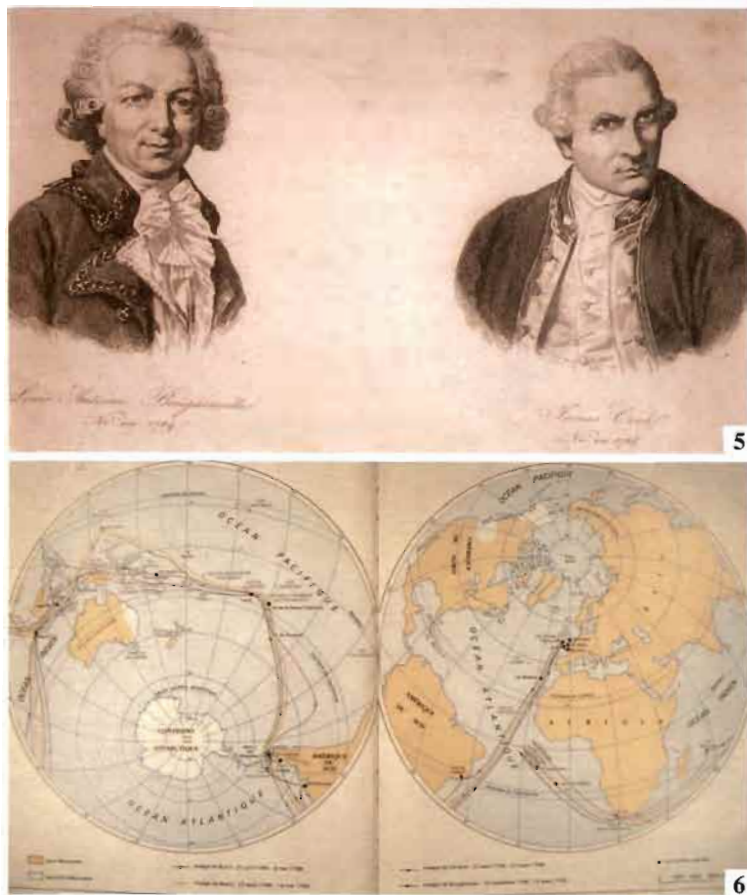


Fig. 4. - Voyages of Mendana (1567), Quiros (1606), Mendana-Quiros (1595), Lemaire et Schouten (1616). Reprinted from Scemla, 1994. Fig. 5. - Portraits of Bougainville and Cook. Photo and Coll.: Bibliothèque du Muséum national d'histoire naturelle, Paris. Fig. 6. - Voyages of Byron (1764-1769) (in green), Wallis (1766-1768) (in yellow), Carteret (1766-1769) (in violet), Bougainville (1766-1769) (in black). Map reprinted from Jacques Brosse "Les tours du Monde des explorateurs" © Bordas, Paris, 1983. Fig. 7. - Fish from Bougainville's expedition. a: Original drawing by Commerson, Skipjack. b: two fish "en herbier", *Halichoeres centriquadra*. Photo and Coll.: Bibliothèque du Muséum national d'histoire naturelle, Paris.



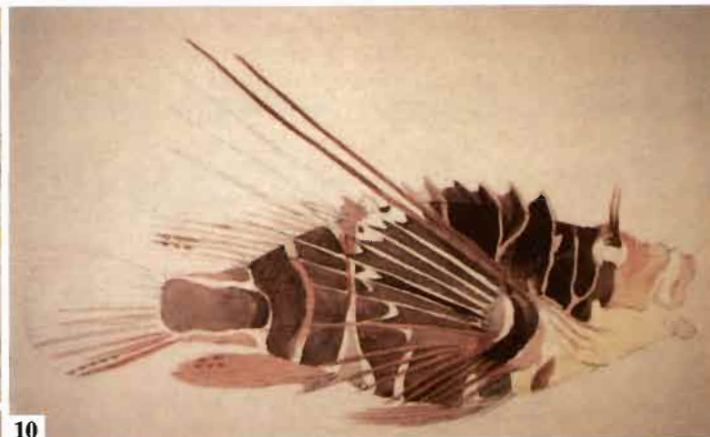
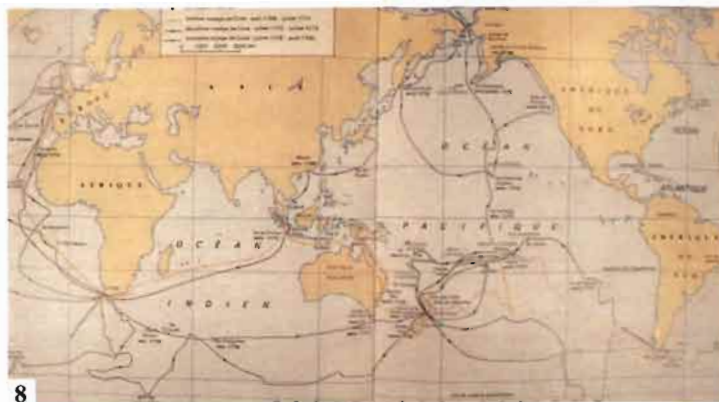


Fig. 8. - The three voyages of Captain Cook: 1768-1771 (in yellow); 1772-1775 (in green); 1776-1780 (in violet). Map reprinted from Jacques Brosse "Les tours du Monde des explorateurs" © Bordas, Paris, 1983. Fig. 9. - Parkinson's drawing of *Megaprotodon strigangulus* (Cuvier, 1831), described by Cuvier in the genus *Chaetodon* from Parkinson's drawing, and a description of a specimen of 120 mm from Otahite made by Solander. Reprinted from Whitehead, 1969. Fig. 10. - Parkinson's drawing of *Pterois radiata* (Cuvier, 1829), described by Cuvier in the genus *Scorpaena* from Parkinson's drawing of fish collected at Otahite. Reprinted from Whitehead, 1969. Fig. 11. - Parkinson's drawing done in 1768 from a fish of 435 mm collected at Quenne Charlotte Sound (Cook Strait, between the two islands of New Zealand) and named *Sciaena mulloides*. It is a specimen of *Aripis trutta* (Bloch & Schneider, 1801). Reprinted from Whitehead, 1969.

## THE NATURALISTS

At the beginning of the XIXth Century, the general configuration of the Pacific was well known, but many details remained unknown. The exact position of the numerous islands and thousands of nautical miles of coastline still had to be established; precise maps still had to be drawn; and observations and studies in all scientific disciplines still had to be completed.

With the end of the Napoleonic Wars which had ravaged Europe, scientists and navigators enjoyed more favourable circumstances. A long period of peace enabled the great European and American powers to promote the advancement of science, and to organize several marine expeditions for this purpose.

They are too numerous for me to mention, but table I provides a summary. A few years ago and in a French journal (now translated into English), I listed the French expeditions in which the great naturalists, who considerably enriched the ichthyological collections of the Muséum national d'histoire naturelle (Paris), had taken part. I think particularly of Jean René Constant **Quoy** and Paul **Gaimard**, who took part in the voyages of Louis Claude **Desaulces de Freycinet** on the *Uranie* (Fig. 16) and Jean Sébastien

Table I. - Oceanic expeditions in the Indo-Pacific posterior to 1815. d = artist.

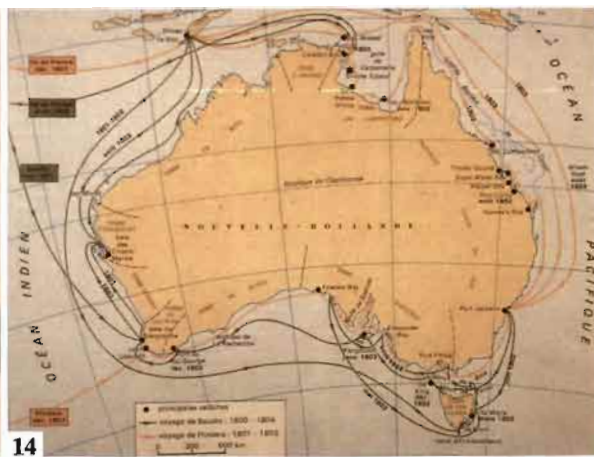
Dates	Ships	Captains	Naturalists and artists
<b>France</b>			
1817-1820	L'Uranie & La Physicienne	L.C. de Freycinet	J.R. Quoy; J.P. Gaimard; J. Arago (d)
1819-1821	Le Rhône & La Durance	P.H. Philibert	G.S. Perottet
1822-1825	La Coquille	L.I. Duperrey	P. Garnot; R.P. Lesson; Lejeune (d)
1824-1826	La Thétis	H. de Bougainville	L. Busseuil
" "	L'Espérance	N. Nourquer du Camper	
1826-1829	L'Astrolabe	J.S.C. Dumont d'Urville	J.R. Quoy; J.P. Gaimard- de Sainson (d)
1827-1828	La Chevette	Th. Fabré	A. Reynaud
1830-1832	La Favorite	P.Th. Laplace	F.T. Eydoux
1836-1837	La Bonite	A.N. Vaillant	F.T. Eydoux; L.F. Souleyet
1836-1839	La Vénus	A.A. Dupetit-Thouars	A. Neboux; Ch.R. Leclancher
1837-1840	L'Astrolabe	J.S.C. Dumont d'Urville	J.B. Hombron; H. Jacquinot
" "	La Zélée	Ch.H. Jacquinot	E.J.F. Le Guillou; J. Grange
1842-1846	Le Rhin	A. Bérard	L. Arnoux; J. Verreaux; Ch. Maryon (d)
1816-1840	Le Buffon & Le Georges Cuvier	J.-J. Dussumier	
<b>England</b>			
1825-1828	Blossom	F.W. Beechey	G.T. Lay
1831-1836	Beagle	R. Fitz-Roy	Ch. Darwin; A. Earle; C. Mertens (d)
1835-1842	Sulphur	F.W. Beachey, E. Belcher	Hinds
<b>Russia</b>			
1815-1818	Rurik	O. von Kotzebue	J.F. Echscholtz; A. von Chamisso; L. Choris (d)
1823-1826	Predpriarte	O. von Kotzebue	J.F. Echscholtz; L. Choris (d)
1826-1829	Seniavine	F.P. Lutke	K.H. Mertens; Von Kittlitz; A. Postels (d)



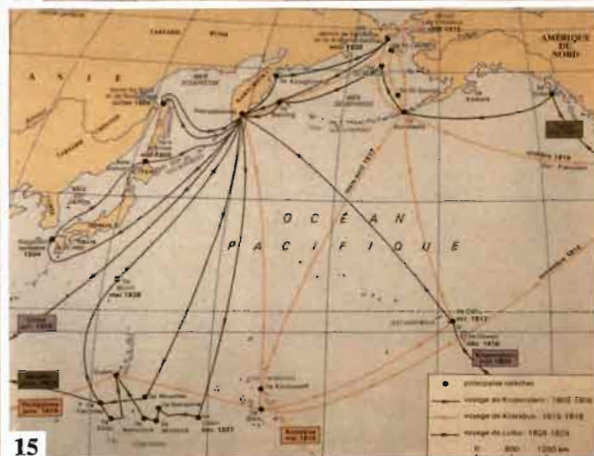
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Fig. 12. - Portraits of La Pérouse and Baudin. Photo and Coll.: Bibliothèque du Muséum national d'histoire naturelle. Fig. 13. - Voyages of La Pérouse (1785-1788) (in yellow) and of d'Entrecasteaux (1791-1794) (in green). Map reprinted from Jacques Brosse "Les tours du Monde des explorateurs" © Bordas, Paris, 1983. Fig. 14. - Voyages of Baudin (1800-1804) (in green) and of Flinders (1801-1803) (in yellow). Map reprinted from Jacques Brosse "Les tours du Monde des explorateurs" © Bordas, Paris, 1983. Fig. 15. - Voyages of Krusenstern (1803-1806) (in violet), of Kotzebue (1816-1818) (in yellow) and of Lutke (1820-1829) (in green). Map reprinted from Jacques Brosse "Les tours du Monde des explorateurs" © Bordas, Paris, 1983.

César **Dumont d'Urville** on the *Astrolabe*. In a work published in 1994, I studied **Quoy's** 281 manuscript pages. All of these were devoted to the fishes from the *Astrolabe* voyage (1826-1829) and are now kept in the library of the Muséum national d'histoire naturelle in Paris, together with 2 notebooks of drawings and 114 unpublished plates. I have been able to identify 996 specimens representing 449 species brought back by this one expedition. Some 211 species were described and illustrated, and an additional 42 were either described or figured by **Quoy** and **Gaimard**, or by **Cuvier** and **Valenciennes**, or by later authors who had ignored the original manuscript descriptions and illustrations. I also think of René Primevère **Lesson** and Prosper **Garnot**, who brought back 288 fish species from the *La Coquille* voyage; of Auguste Adolphe Marc **Reynaud**, whose harvest numbered more than 300 fishes; and of Joseph Fortuné Théodore **Eydoux**, who with Louis François Auguste **Souleyet**, collected more than 200 species.

The naturalists on the English expeditions were also making their contribution. On board the *Blossom* (1825-1827), Frederick William **Beechey** (Fig. 17), himself a competent geographer, draughtsman and zoologist, was assisted by the botanist Georg **Tridescant Lay**. He explored islands of the Gambier group and described numerous species of butterflyfish in the waters of Tuamotu. On board the *Beagle* and the *Adventure*, and under Robert **Fitz-Roy** (Fig. 17), the naturalist was a young 22 year-old student. He had replaced the botanist John Stevens **Henslow** who, being unable to sail, was to write « I do not consider you as a fully fledged naturalist, but I know that you are able to collect and to note what is worthy of being recorded in natural history ». The young naturalist, Charles **Darwin**, would later write that this expedition was the most important event in his life, and that it determined his whole career. With the curiosity and enthusiasm of an amateur, he studies the adaptation of species to their environment, the relations between animals and plants, and the interdependence of species, all of which would become the major themes of his future works - his theories on speciation and natural selection. Let me also mention the long voyage of the *Sulphur*, during which Edward **Belcher** (Fig. 17) made a vast collection, including more than 200 fishes from New Guinea.

In spite of all this progress, the problem of the Southern Continent remained unresolved and still stirred the curiosity of scientists and navigators (Table II). In 1819, two Russians, Fabian Gottlieb von **Bellinghausen** and M. **Lazareff**, reached 69°S and

Table II. - Post 1815 oceanic expeditions looking for the Antarctic continent.

Countries	Dates	Ships	Captains	Areas
Russia	1819-1821	Vostok; Mirny	F. Bellinghausen; M. Lazareff	69° S et 91° W. Iles Pierre- le-Grand et Alexandre Ier
England	1819	Williams	W. Smith	62°30' S. Nouvelle-Bretagne du Sud
England	1820	Andromache	E. Bransfield	64°30' S. Sbetland du Sud
U.S.A.	1820-1821	Hero	N.B. Palmer	62° S. Pointe Palmer
England	1823	Jane	J. Weddell	Orcades du Sud et 74° S.
England	1831	Tula; Lively	J. Biscoe; G. Avery	68° S. Terres d'Enderby et de Graham
France	1837-1840	Astrolabe; Zélée	J.S.C. Dumont d'Urville; Ch.H. Jacquinot	67° S. Terre-Adélie
U.S.A.	1836-1842	Vincennes; Flying Fish	Ch. Wilkes; W.M. Walkers	69° S. Terre de Wilkes
England	1839-1843	Erébus; Terror	J.C. Ross; F. Crozier	76° S. Ile Franklin - 78° S. Ile Ross; Volcans Erebus & Terror



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BAUCHOT

Fig. 16. - Voyages of Freycinet (1817-1820) (in violet), of Duperrey (1822-1825) (in green) and of Dumont d'Urville (1826-1829) (in yellow). Map reprinted from Jacques Brosse "Les tours du Monde des explorateurs" © Bordas, Paris, 1983. Fig. 17. - Voyages of Bechey (1825-1828) (in violet), of Fitz-Roy (1831-1836) (in yellow) and of Belcher (1836-1842) (in green). Map reprinted from Jacques Brosse "Les tours du Monde des explorateurs" © Bordas, Paris, 1983. Fig. 18. - Portrait of Dumont d'Urville. Photo and Coll.: Bibliothèque du Muséum national d'histoire naturelle, Paris.





Fig. 19. - Voyages of Dumont d'Urville (1837-1840) (in yellow), of Wilkes (1838-1842) (in violet) and of Ross (1839-1843) (in green). Map reprinted from Jacques Brosse "Les tours du Monde des explorateurs" © Bordas, Paris, 1983.

were probably the first men to see the Antarctic Continent when they discovered Peter the Great and Alexander the First Islands. In the same year, the Englishman **William Smith** rounded Cape Horn and discovered an unknown land he called South New Britain. In 1820, a countryman of his, **Edward Bransfield**, reconnoitered the neighbouring South New Shetland Islands. In 1820-1821, an American, **Nathaniel Brown Palmer**, sighted a long mountainous point but could not land. It was the Palmer Peninsula. In 1823, **James Weddell**, another Englishman, reached 74°S and discovered the South Orkneys. In 1831, **John Briscoe** and **George Avery** plotted the positions of Enderbyland and Grahamland.

In the search for the Antarctic Continent, one person who stands out is **Dumont d'Urville** (Fig. 18). He completed his first voyage round the World as Second-in-Command under **Louis Isidore Duperrey** aboard *La Coquille*. During his second voyage (1826-1829) in command of *L'Astrolabe*, he took bearings of more than 2,500 nautical miles of often unknown or inaccurately sited coasts, and discovered or rectified the exact positions of more than 150 islands. Thanks to the information obtained from the Englishman **Peter Dillon** in Tukopia in 1826, he had also found **La Pérouse's** trail in Vanikoro. Finally, he gathered an enormous amount of ethnographic, zoological and botanical data.

Moved by a passion for discovery, as **Cook** had been before him, and in spite of his poor health, **Dumont d'Urville** offered his services for an expedition organized to discover the South Pole. He sailed once more in 1837 on board *L'Astrolabe* and *La Zélée* (Fig. 19). On 19 January 1840, he discovered the coasts of the Antarctic Continent, which he called Terre-Adélie in honour of his wife. He was also able to determine the approximate position of the magnetic pole. When he came back to Toulon in November 1840, he had successfully completed the most astonishing of all the voyages of discovery ever attempted under the French flag. During his voyage he had drawn more than 100 sea charts, the quality of which was so remarkable that they remained unaltered until after the Second World War. The natural history collections he brought back were the richest ever gathered by a single expedition. They include among other things, more than 400 fishes.

During his circumnavigation **Dumont d'Urville** had passed **Charles Wilkes'** expedition (1838-1842) (Fig. 19). The American, with 6 ships and 12 scientists, had been instructed to assert American rights in the Southern Hemisphere. But the venture had been badly prepared and when it ended, three of the six ships and 124 men had been lost.

Although the English were the last to start their exploration of Antarctica, their voyage was much better prepared. On board the *Erebus* and *Terror* and during the years between 1839 and 1843, **James Clark Ross**, who had become an accomplished zoologist, sailed round the greater part of the Antarctic Continent and explored its coasts (Fig. 19). He landed on Franklin Island at 76°S, then on Ross Island at 78.4°S and named the two volcanoes Erebus and Terror. He also took the first abyssal sounding (4,850 m) and noted the temperatures at different depths. In short, this work was the forerunner to the science of oceanography. He also brought back, thanks to **McCormick's** and **Robertson's** help, more than 300 fish species. At that time, none were known from farther south than 50°S.

The time of the great maritime explorations in the Pacific Ocean virtually ends with **Dumont d'Urville, Wilkes** and **Ross**. Efforts spanning three centuries had been necessary to explore the largest ocean in the World and to determine the extent and richness of its natural resources. The publications based on all these expeditions enable us to appreciate their scientific value. Between 1815 and 1850, the French published 85 volumes of observations (not including the atlases). Forty-two of these were devoted solely to **Dumont d'Urville's** voyages. The Russians published 17 works, the English 13 and the Americans 9.

These great expeditions, during which science was never forgotten, have had long-term consequences because they were the foundation on which great syntheses and all-embracing theories, like **Darwin's**, were developed. They could never have been achieved without the patient labour of naturalists who, at the risk of life and limb, continued to collect, describe and illustrate. Not only was the Indo-Pacific area explored and its human populations encountered, but its flora and fauna were made known to science.

**Acknowledgements.** - I would like to thank Butch and Jane Hulley warmly for the English translation of this lecture presented in French at the Conference.

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## ZOOGEOGRAPHY OF CORAL REEF FISHES OF THE INDO-PACIFIC REGION

by

John E. RANDALL (1)

The vast tropical and subtropical Indo-Pacific region extends from the Red Sea and east coast of Africa to the Hawaiian Islands and Easter Island. Within the Indo Pacific, clearly the richest fish fauna is found in the Indo-Malayan region (southeast Asia, East Indies, and northern Australia). Within this area, Indonesia and the Philippines are estimated to have 2,900 species of reef and shore fishes. One reason for the rich diversity of species is the stability of the region with respect to temperature during an ice age. Extinction rates were high in the higher latitudes and on the eastern side of the three oceans with tropical biota where cool currents compress the tropical zone. By contrast, the west side with a broader tropical zone, was affected less by ice age temperature drop.

Another reason is the result of a barrier to east-west dispersal of a large percentage of reef and shore fishes during an ice age. Over the last 700,000 years there have been at least three (but perhaps as many as six) glacial periods capable of lowering the sea level enough to close the Torres Strait, the Malacca Strait, and the Sunda Strait (between Sumatra and Java). However, there was not a total east-west land barrier. What made it a total barrier to east-west dispersal of purely marine species was the low salinity and high turbidity resulting from discharge of large rivers in the area, coupled with postulated upwelling of cooler water from the larger land masses. Thus we have the potential, with each ice age (the last was 18,000 years ago) to double the number of species in the area, hence a scenario comparable to the land barrier that occurred in Panama during the last glacial period that produced the geminate fish species of Jordan (i.e., closely related Caribbean and eastern Pacific species pairs). When an interglacial period followed and sea level rose, the isolated faunas could intermix. Some incipient species merged, whereas others remained as distinct sister species. Many examples of such species pairs are known, such as *Chaetodon lineolatus* and *C. oxycephalus* (Pl. IA, B), *Amphiprion percula* and *A. ocellaris* (Pl. IC, D), and *Siganus doliaus* and *S. virgatus* (Pl. IIA, B), but perhaps there are others that may have differentiated ecologically, behaviourally, or physiologically, but not enough morphologically for us to readily detect.

Springer and Williams (1990) provided examples of species with distributions in the Pacific and Indian Oceans but without any records for the Indo-Malayan region. They postulated that such species may have become extinct in the Indo-Malayan region during a glacial period and not been able to reinvade later. Perhaps these are species for which some ecological requirement is needed to complete their life history, such as clear oceanic water for the development of larvae. Or in this richest area there may now be too much competition for them to resume their niche. Or perhaps there are now predators, parasites, or diseases which preclude their recolonization.

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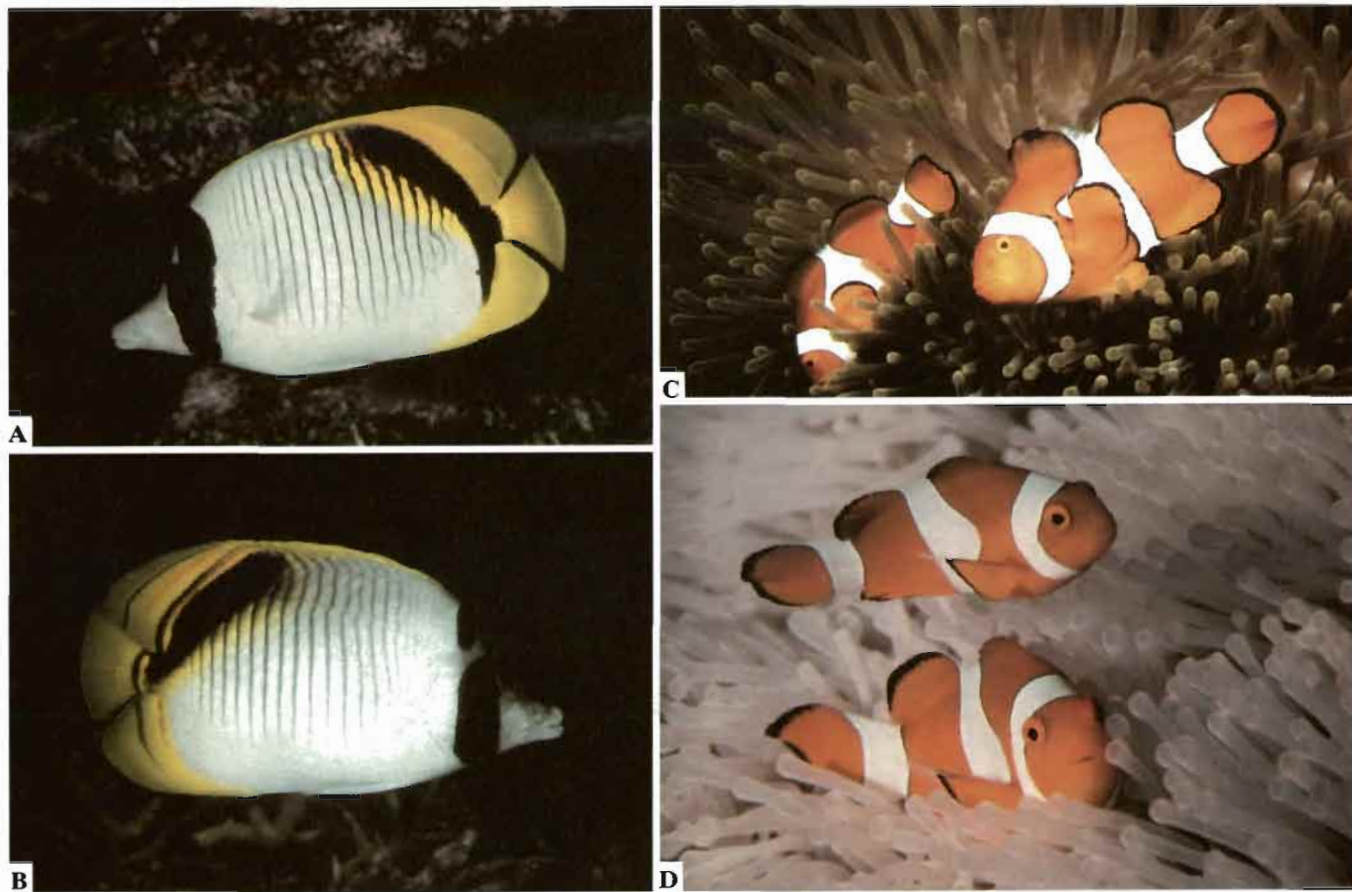


Plate I. - Examples of pairs of sister species. A: *Chaetodon lineolatus* (Fanning Is.); B: *Chaetodon oxycephalus*, 150 mm (Palau); C: *Amphiprion percula*, 75 mm (d'Entrecasteaux Is., Papua New Guinea); D: *Amphiprion ocellaris*, 50 mm (Stephanie Is., Indonesia)



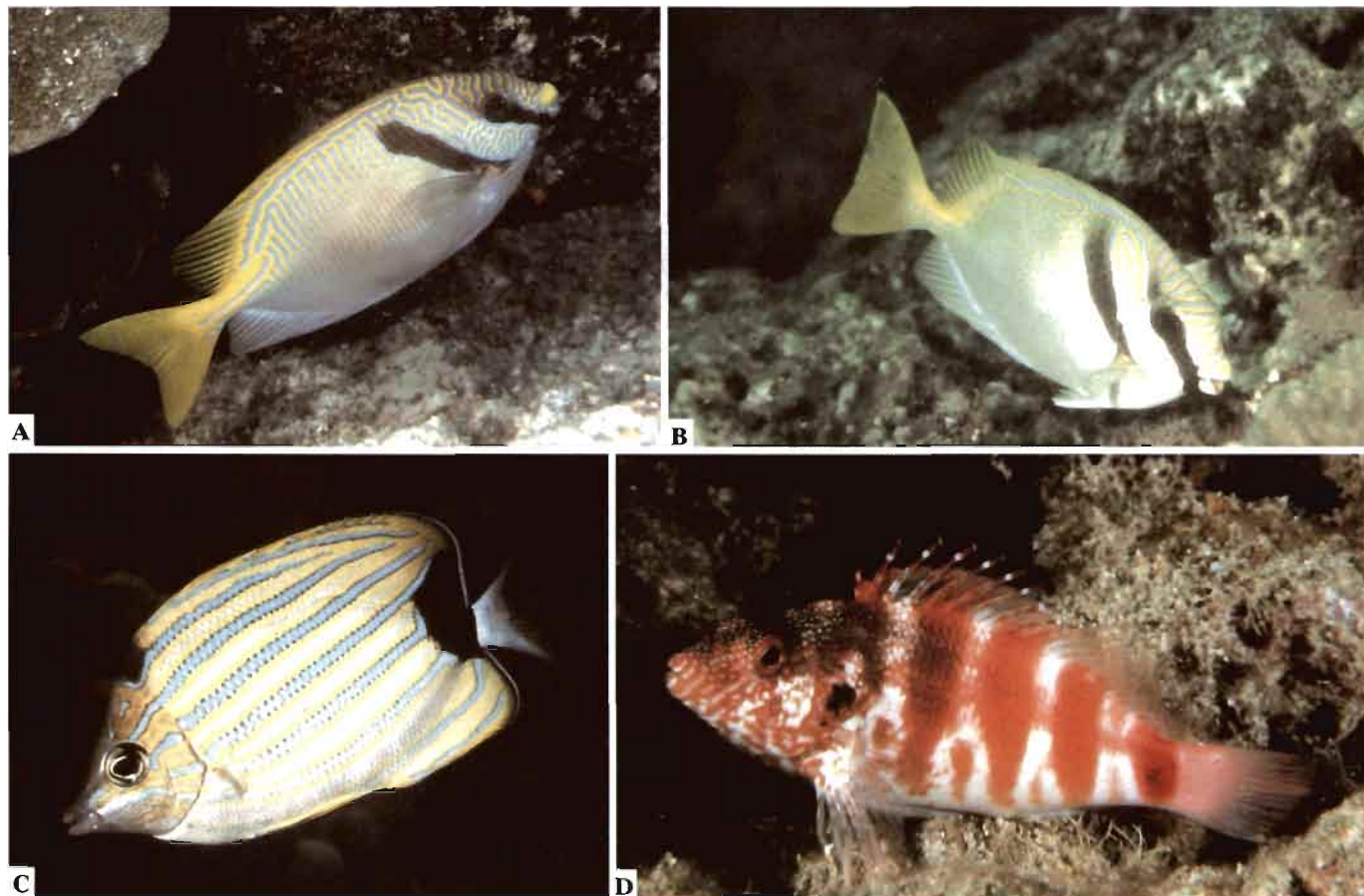


Plate II. - Examples of pairs of sister species. **A:** *Siganus doliaus*, 170 mm (Heron Is.); **B:** *Siganus virgatus* (Sri Lanka); **C:** *Chaetodon fremblii* (Oahu); **D:** *Cirrhitops fasciatus* (Oahu).

We also have north-south disjunct populations in the western Pacific termed anti-tropical and antiequatorial. The same explanations could apply as the east-west dislocations, plus the obvious possibility of these being species adapted to subtropical temperatures, hence unable to survive in low latitudes during interglacial periods.

The principal Indo-Pacific areas of endemism other than the Indo-Malayan region are the Red Sea; Natal; Madagascar, Réunion and Mauritius; southern Oman; Andaman Sea; western Australia; southern Japan; southeastern Australia; Lord Howe Island and Norfolk Island; Hawaiian Islands; Marquesas Islands; Pitcairn Group and Rapa; and Easter Island. Of these, the reef and shore fishes of the Hawaiian Islands have the highest percentage of endemism, 23.1%, but closely followed by Easter Island with 22.2%. These percentages change as our knowledge of the faunas increases. Gosline and Brock (1960) determined the endemism for reef fishes in Hawaii as 34%. The discovery of alleged Hawaiian endemics elsewhere and the reporting of new records of wide-ranging Indo-Pacific species to Hawaii (some apparently only as waifs) have lowered the number of endemic species.

Some authors believe that speciation can only be the result of a vicariant event, such as the separation of the western Atlantic and eastern Pacific marine biota with the emergence of a land barrier in Panama during a glacial period. However, it seems clear for the highly isolated islands, such as those of the Hawaiian Islands and Easter Island, that speciation has occurred as a result of the chance colonization by larvae, followed by a long period of little or no gene flow. No vicariant event is needed. A successful spawning by the progenitor stock, coupled with just the right current pattern (such as entering a gyre at just the right time) could result in a pulse of larvae to an area not reached before and not likely to be attained again in the near future.

The sister species of some of the Hawaiian and Easter Island endemics can be readily identified. Others are clearly relics for which no closely related species are known. Examples are the scorpionfish *Pterois sphex*, the grouper *Epinephelus quernus*, the butterflyfish *Chaetodon fremblii* (Pl. IIC), the angelfishes *Centropyge potteri* and *Genicanthus personatus*, the wrasse *Coris flavovittatus*, and the parrotfish *Chlorurus perspicillatus*. The Hawaiian Islands have been forming intermittently over a fixed volcanic site in the Pacific Plate for at least 70 million years, so there has been ample time for a species to become extinct except in Hawaii where the cause of extinction elsewhere, such as more efficient predatory or competitor species, did not happen. A unique example of a relic species is the hawkfish *Cirrhitops fasciatus* (Pl. IID) known only from the Hawaiian Islands, Mauritius, Réunion and Madagascar.

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## Symposium 1

### FRESH AND BRACKISH WATER FISHES

Chairperson: **Tyson ROBERTS**

- MARQUET G. & N. MARY. - Comments on some New Caledonian freshwater fishes of economical and biogeographical interest. 29-39  
*Remarques sur quelques poissons d'eau douce de Nouvelle-Calédonie présentant un intérêt économique ou biogéographique.*
- MARQUET G., MARY N. & R. WATSON. - Comments on the freshwater fish-fauna of French Polynesia. 41-44  
*Remarques sur l'inventaire des poissons d'eau douce de Polynésie.*
- MARTIN-SMITH K., BULLOUGH L. & L. LAIRD. - Short-term movements of freshwater fishes in Sabah, Malaysia. 45-56  
*Mouvements à court terme de poissons d'eau douce à Sabah, Malaisie.*
- JANEKITKARN S., PREMCHAREON S. & P. VITHEESAWAT. - Estuarine fishes of the Tha-Chin River, Samut Sakorn, Thailand. 57-64  
*Les poissons estuariens de la rivière Tha-Chin, Samut Sakorn, Thaïlande.*
- SONTIRAT S. - Description of a new species of *Acantopsis* (Cypriniformes: Cobitidae) from Thailand. 65-68  
*Une nouvelle espèce d'Acantopsis (Cypriniformes: Cobitidae) de Thaïlande.*
- MEISNER A.D. & B.B. COLLETTE. - Generic relationships of the internally-fertilized Southeast Asian halfbeaks (Hemiramphidae: Zenarchopterinae). 69-76  
*Relations phylétiques des demi-becs (Hemiramphidae: Zenarchopterinae) à fécondation interne du Sud-Est Asiatique.*



## COMMENTS ON SOME NEW CALEDONIAN FRESHWATER FISHES OF ECONOMICAL AND BIOGEOGRAPHICAL INTEREST

by

Gérard MARQUET & Nathalie MARY (1)

**ABSTRACT.** - Since 1991, regular surveys of the New Caledonian freshwater fishes have been carried out in order to better understand the ecology of this insular fishfauna. A number of these fishes have some economical importance, such as the eels (Anguillidae), the flagtails (Kuhliidae) and the mullets (Mugilidae). Other fishes present great biogeographical interest such as the relic species (*Galaxias neocaledonicus*) and some gobies (Gobiidae) which are the most successful invaders of freshwaters of the Indo-Pacific islands.

**RÉSUMÉ.** - Remarques sur quelques poissons d'eau douce de Nouvelle-Calédonie présentant un intérêt économique ou biogéographique.

Depuis 1991, des missions régulières ont été effectuées pour mieux comprendre l'écologie des poissons d'eau douce de Nouvelle-Calédonie. Certains de ces poissons ont une importance économique, tels que les anguilles (Anguillidae), les "carpes" ou doules (Kuhliidae) et les mulets (Mugilidae). D'autres poissons présentent un grand intérêt biogéographique, tels le poisson relique, *Galaxias neocaledonicus*, et certains gobies ou lochons (Gobiidae) qui sont les envahisseurs dominants dans les eaux douces des îles indo-pacifiques.

**Key-words.** - Anguillidae, Galaxiidae, Gobiidae, Kuhliidae, Mugilidae, New Caledonia, Freshwater, Biogeography, Inventory.

New Caledonia (Fig. 1) extends between 164°-168°E and 20°- 23°S with an area of 19,500 km<sup>2</sup> comprising 8 islands, with the largest, New Caledonia, locally called "La Grande Terre". Freshwater fishes of New Caledonia were first studied by Weber and de Beaufort in 1915 who recorded 30 species belonging to 11 families. Catala (1950) reported on a number of fishes with some economical interest. In 1991, a two month freshwater survey of "La Grande Terre" (PEDCAL Project) was conducted from September to October (Séret, 1992). As a result of this survey, descriptions of new species (Dingerkus and Séret, 1992b, 1992c), new records (Dingerkus and Séret, 1992a), and inventories (Marquet, 1996; Marquet *et al.*, 1997; Séret, 1997) have been published.

Further collections were made in 1996-97, to investigate the ecology of this fish-fauna, in conjunction with a study on biotic indices (Mary, 1996). As a result of the past and recent field studies, 64 sites located on 33 rivers and one lake were sampled (Fig. 1). These studies allowed to complete the inventory and today about 80 species, representing 33 families, have been recorded in New Caledonian fresh waters (Marquet *et al.*, 1997).

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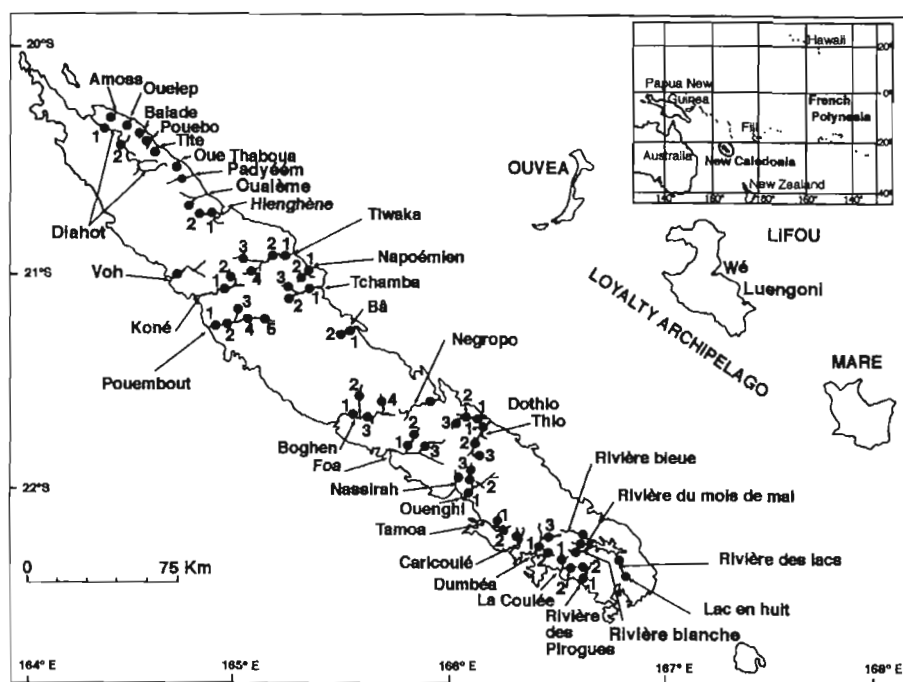


Fig. 1. - Map of New Caledonia showing the survey sites and the corresponding rivers.

Some of these fishes having some economical importance and/or biogeographical interest are herein presented.

## MATERIALS AND METHODS

The fish were mainly collected by electrofishing equipment, and occasionally rotenone was also used. The sampling sites of the different surveys are listed in Appendix 1 and shown on figure 1.

PEDCAL is the acronym for "Poissons d'Eau Douce de Nouvelle-Calédonie", and designates the program of researches carried out in 1991 (Séret, 1992).

The specimens have mainly been deposited in the collections of the Muséum national d'histoire naturelle, Paris (MNHN).

## RESULTS

### Fishes of economical interest

Fishes of the 3 following families, Anguillidae, Kuhliidae and Mugilidae, are traditionally consumed by local people (Catala, 1950) and they still have some commercial and recreational importance (Séret, 1992).

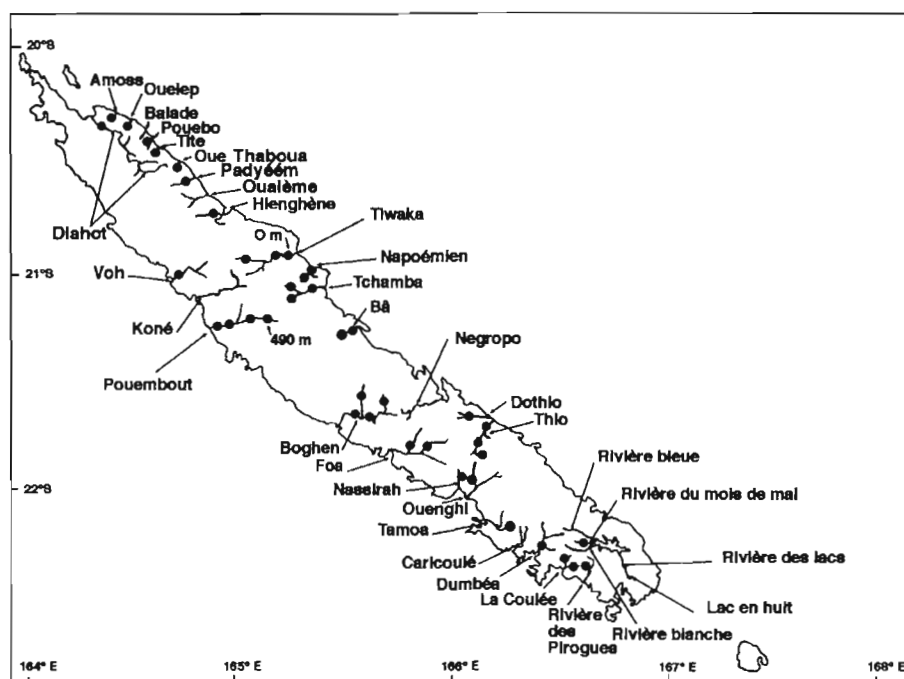


Fig. 2. - Distribution of *Anguilla marmorata* in New Caledonia.

#### *Anguillidae (Eels)*

Five species of eels occur in New Caledonia (Marquet, 1996): *Anguilla australis schmidti* Philipps, 1925; *A. marmorata* Quoy & Gaimard, 1824; *A. megastoma* Kaup, 1856; *A. obscura* Günther, 1871, and *A. reinhardtii* Steindachner, 1867.

*A. marmorata* (Fig. 2) and *A. reinhardtii* (Fig. 3) are ubiquitous, their range extending from the upper to the lower reaches, in flowing waters. Large specimens of the former are often found on the east coast and the latter on the west coast.

*A. obscura* and *A. megastoma* are less abundant than the above mentioned species (Fig. 4). *A. obscura* is represented mainly by small specimens. Marquet (1996) has recorded two small specimens in Tiwaka river (Stations PEDCAL 43 and 44). The species is widespread present only in lower reaches of rivers. *A. megastoma* is usually represented by large specimens and is found only in middle and upper reaches of rivers.

*A. australis schmidti* (Fig. 5) is widely spread but only small specimens have been captured. Marquet (1996) lists two small specimens from Tiwaka river (Station PEDCAL 43) and La Foa river (Station PEDCAL 23). The fish appear to occur in still waters; possibly larger specimens may occur in swamps as may larger specimens of *A. obscura* as this is the case in French Polynesia (Marquet and Galzin, 1991).

#### *Kuhliidae (Flagtails)*

Three species of flagtails occur in New Caledonia: *Kuhlia marginata* (Cuvier, 1829), *K. munda* (De Vis, 1885) and *K. rupestris* (Lacepède, 1802). *K. munda* is widespread in the territory but is restricted to brackish water. *K. marginata* (Fig. 6), locally named

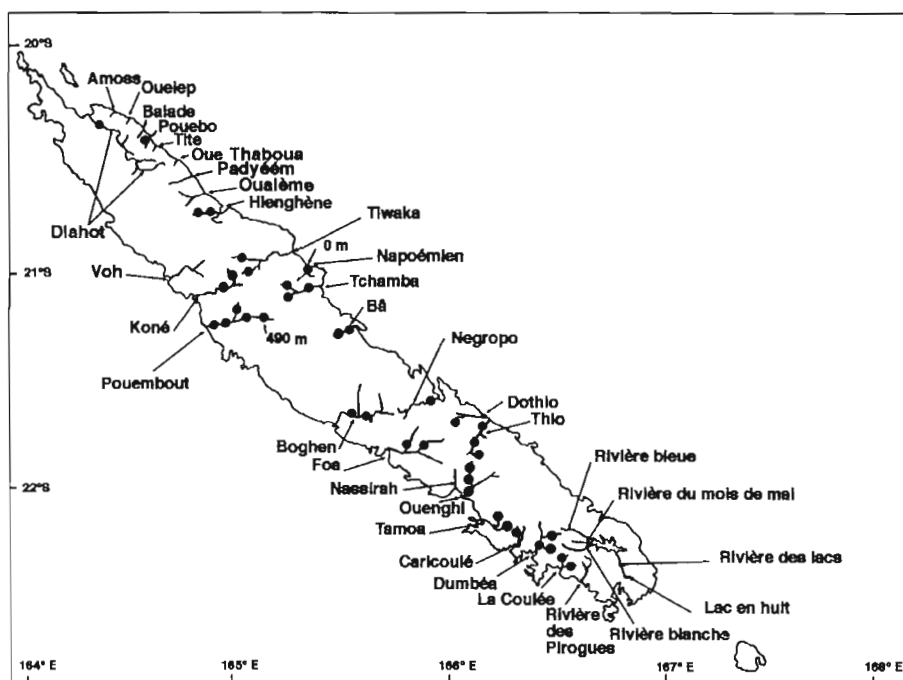


Fig. 3. - Distribution of *Anguilla reinhardtii* in New Caledonia.



Fig. 4. - Distribution of *Anguilla obscura* and *A. megastoma* in New Caledonia.

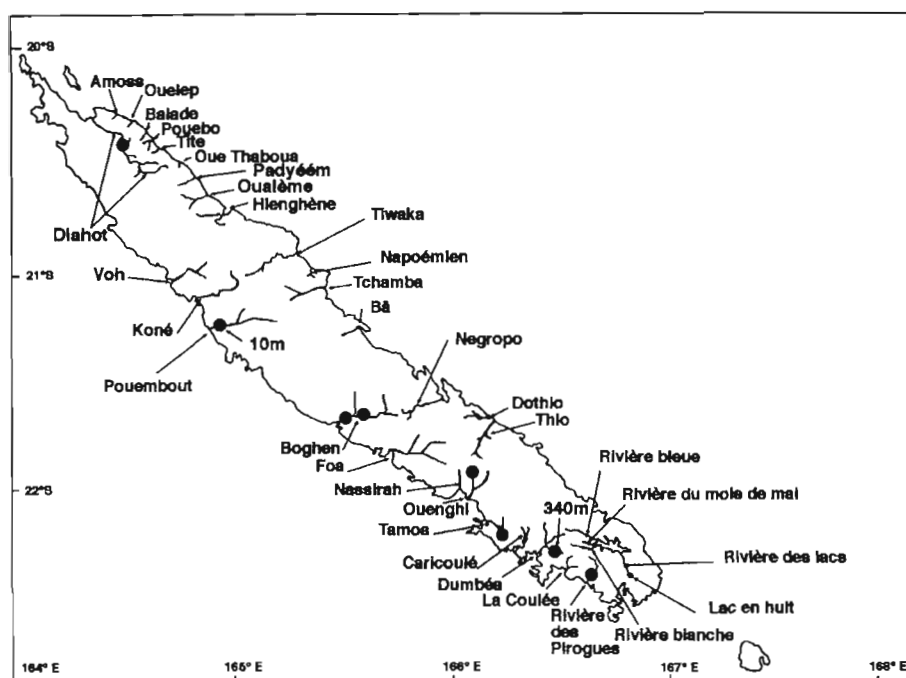


Fig. 5. - Distribution of *Anguilla australis schmidii* in New Caledonia.

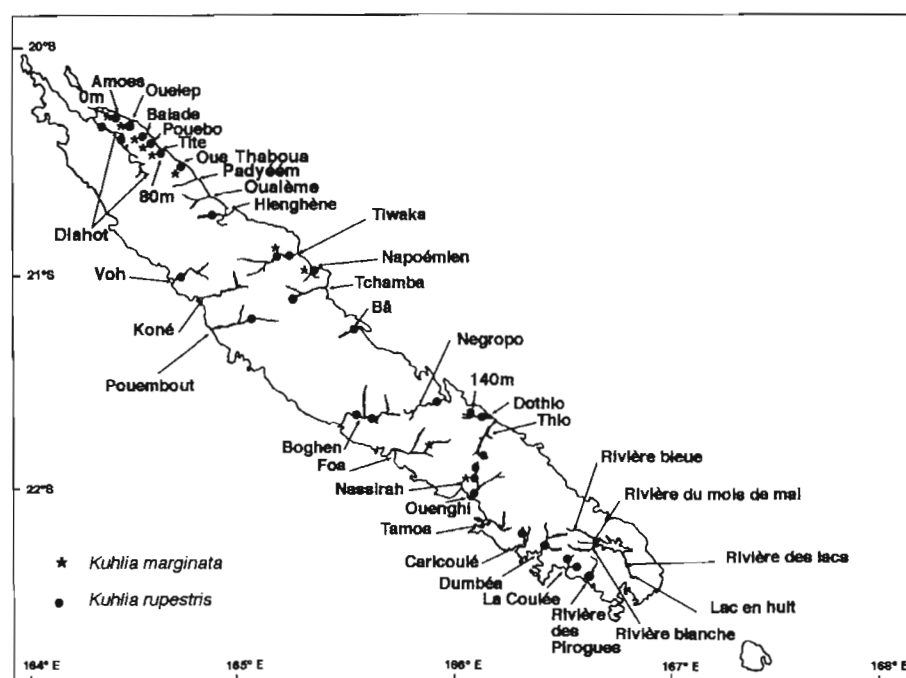


Fig. 6. - Distribution of *Kuhlia marginata* and *K. rupestris* in New Caledonia.

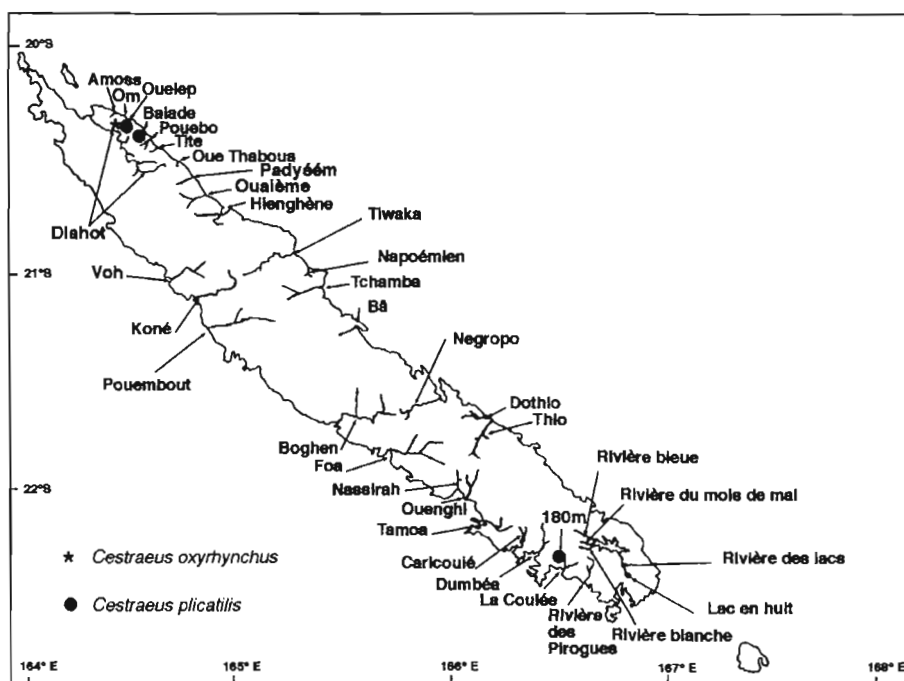


Fig. 7. - Distribution of *Cestraeus oxyrhynchus* and *C. plicatilis* in New Caledonia.

"Queue rouge" (redtail), is confined to the lower reaches of rivers of the east coast. The most common, *K. rupestris*, locally named "Carpe", is found in the lower and middle reaches of most rivers, where it is sometimes abundant.

#### *Mugilidae* (Mulletts)

Two species of mullets are present in New Caledonia: *Cestraeus oxyrhynchus* Bleeker, 1855 and *C. plicatilis* Valenciennes, 1836. They are locally named "Mulets noirs" (black mullets) and strongly appreciated. These two phytophagous species occur from the middle to lower reaches of flowing rivers, because they are unable to climb waterfalls. Both species are uncommon and have only been found in three rivers (Fig. 7).

#### Fishes of biogeographical interest

Most galaxiids are confined to temperate and cold temperate freshwaters of southern hemisphere, except for the most tropical which occurs in New Caledonia: *Galaxias neocaledonicus* Weber & de Beaufort, 1913. This species is considered an indicator of affinities between the ichthyofauna of New Zealand and New Caledonia (Mc Dowall, 1990) or Australia and New Caledonia (Séret, 1997). *G. neocaledonicus* was known to be confined to "Lac en huit" (Eight-shaped lake) and "Grand Lac" (Great lake) in southern New Caledonia, but it has recently been found in rivers close to "Lac de Yaté" (Yaté lake): i.e. "Rivière bleue" (Blue river), "Rivière du mois de mai" (River of May), "Rivière des lacs" (River of lakes) and "Rivière blanche" (White river), also in southern New Caledonia (Fig. 8).



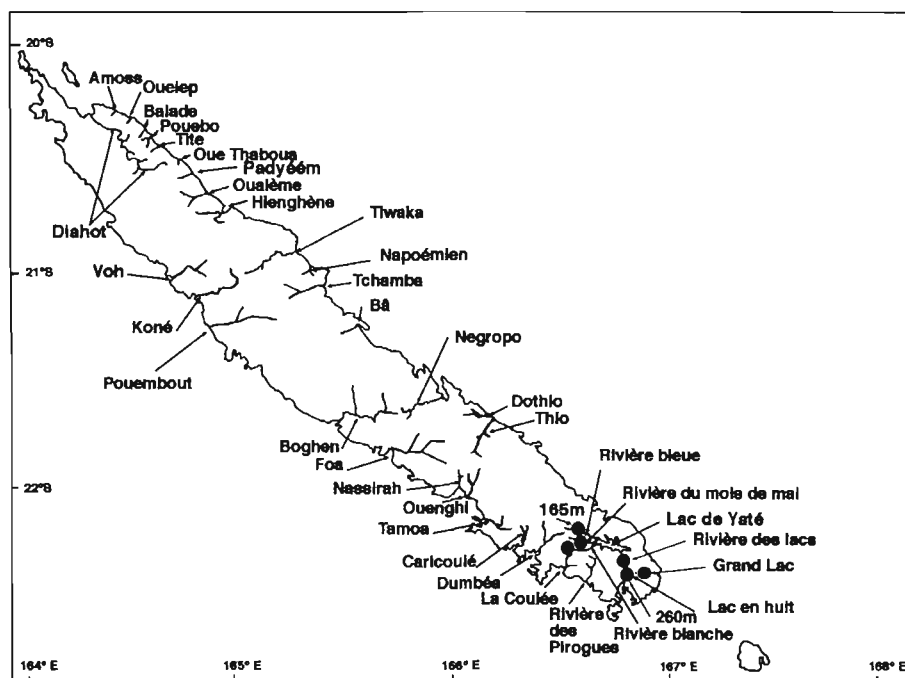


Fig. 8. - Distribution of *Galaxias neocaledonicus* in New Caledonia.

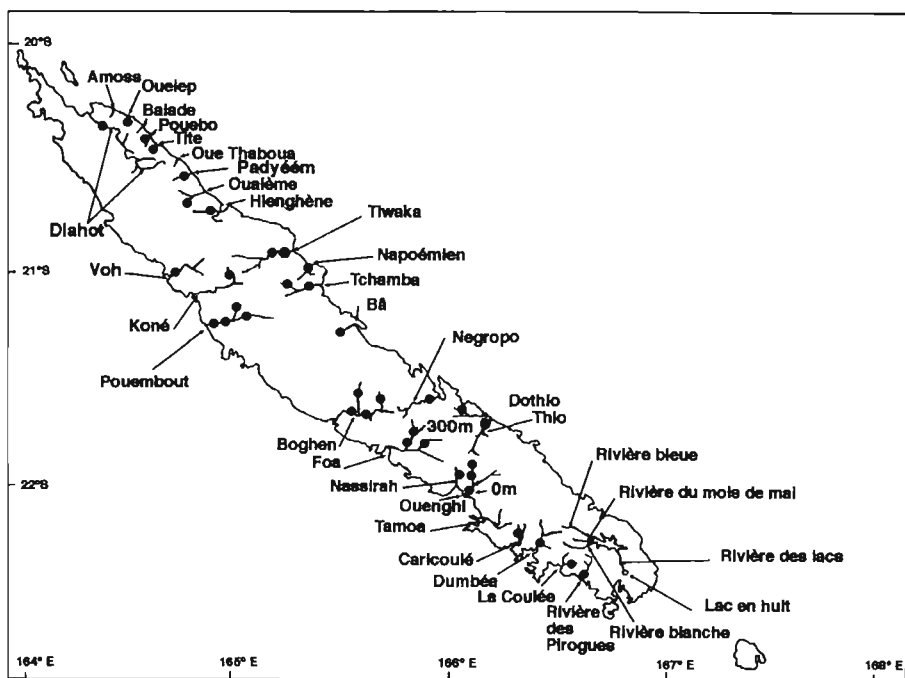


Fig. 9. - Distribution of *Awaous guamensis* in New Caledonia.

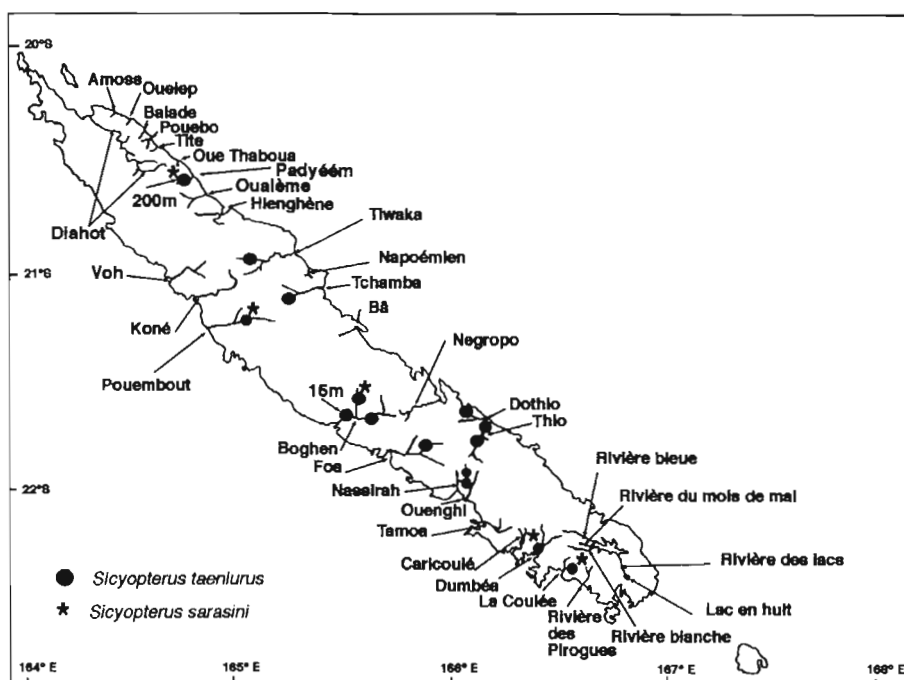


Fig. 10. - Distribution of *Sicyopterus taeniurus* and *S. sarasini* in New Caledonia.

### Invaders

At least 18 species (24.6%) of gobiids occur in New Caledonian waters from the lower to the upper courses of rivers (Marquet *et al.*, 1997). The success of gobies in colonising the Indo-Pacific islands has been mentioned by Ryan (1991). The distribution of three of them is herein commented.

*Awaous guamensis* (Valenciennes, 1837) is by far the most common species along the entire length of most rivers (Fig. 9); a typical distribution for this species is reported by Watson (1992). This goby preys on insects of the families Simuliidae, Hydropsychidae and Leptophlebiidae (NM, pers. obs., 1997) which are ubiquitous and may explain why this goby is widespread in New Caledonian streams.

Two species of *Sicyopterus* are sympatric: *Sicyopterus taeniurus* (Günther, 1877) and *S. sarasini* Weber & de Beaufort, 1915. They occur from the upper to the lower reaches, in flowing waters. *S. taeniurus* is the most common (Fig. 10) and *S. sarasini* is endemic and has only been found in five rivers (Fig. 10).

### CONCLUSION

The ichthyological survey is going on, in conjunction with a study on the distribution of macro-invertebrates (mainly insects and crustaceans) and on an evaluation of the biotic indices. The results of these studies should lead to a better understanding of the ecology of the New Caledonian freshwaters.

**Acknowledgements.** - Sincere thanks are due to Walter Ivanstoft (Macquarie University) for his meaningful comments and to Bernard S  ret (ORSTOM) who initiated the recent surveys in New Caledonian freshwaters and for his help in preparing the manuscript.

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Appendix 1. - List of selected species of Anguillidae, Kuhliidae, Gobiidae, Mugilidae and Galaxiidae, by sampling sites, collected during the freshwater surveys in New Caledonia (PEDCAL, 1991 and field studies of 1996-97).

Rivers	Stn	Altitude	Date	Species
Amoss	-	0 m	22 Sep. 1991	<i>Anguilla marmorata</i> , <i>Kuhlia marginata</i> , <i>K. rupestris</i> .
	1	5 m	14 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> .
Bâ	2	140 m	14 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Awaous guamensis</i> .
Balade	-	30 m	22 Sep. 1991	<i>Cestraeus plicatilis</i> , <i>Kuhlia marginata</i> , <i>K. rupestris</i> .
Boghen	1	15 m	07 Jul. 1996	<i>Anguilla australis schmidtii</i> , <i>A. marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> .
	2	80 m	07 Jul. 1996	<i>Anguilla marmorata</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> , <i>S. sarasini</i> .
	3	45 m	06 Jul. 1996	<i>Anguilla marmorata</i> , <i>A. australis schmidtii</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> .
	4	250 m	06 Jul. 1996	<i>Anguilla marmorata</i> , <i>Awaous guamensis</i> .
Caricoué	-	22 m	01 Oct 1991	<i>Anguilla reinhardtii</i> , <i>Awaous guamensis</i> , <i>Kuhlia rupestris</i> .
Diahot	1	40 m	21 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> .
	2	60 m	21 Sep. 1991	<i>Anguilla australis schmidtii</i> , <i>Kuhlia rupestris</i> .
Dothio	1	20 m	27 Sep. 1991	<i>Kuhlia rupestris</i> .
	2	140 m	28 Jul. 1996	<i>Anguilla marmorata</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> .
	3	580 m	27 Sep. 1991	<i>Anguilla megastoma</i> , <i>A. reinhardtii</i> .
Dumbéa	1	40 m	08 Jun. 1996	<i>Anguilla marmorata</i> , <i>A. obscura</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> , <i>S. sarasini</i> .
	2	340 m	13 Sep. 1991	<i>Anguilla australis schmidtii</i> , <i>A. reinhardtii</i> .
	3	150 m	07 Sep. 1991	<i>Anguilla reinhardtii</i> .
Huenghène	1	25 m	24 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> .
	2	45 m	24 Sep. 1991	<i>Anguilla reinhardtii</i> .
Koné	1	80 m	25 Sep. 1991	<i>Anguilla reinhardtii</i> .
	2	105 m	25 Sep. 1991	<i>Anguilla reinhardtii</i> , <i>Awaous guamensis</i> .
La Coulée	1	20 m	30 Jun. 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Cestraeus plicatilis</i> .
	2	50 m	15 Jun. 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> , <i>S. sarasini</i> .
La Foa	1	60 m	11 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Awaous guamensis</i> .
	2	300 m	11 Sep. 1991	<i>Awaous guamensis</i> .
	3	50 m	06 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>A. obscura</i> , <i>Kuhlia marginata</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> .
Lac en huit	-	260 m	17 Sep. 1991	<i>Galaxias neocaledonicus</i> .
Napoémien	1	0 m	15 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia marginata</i> , <i>K. rupestris</i> , <i>Awaous guamensis</i> .
	2	200 m	15 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. megastoma</i> .
Nassirah	-	125 m	10 Jun. 1997	<i>Anguilla marmorata</i> , <i>A. megastoma</i> , <i>Awaous guamensis</i> .
Negropo	-	50 m	11 Sep. 1991	<i>Anguilla obscura</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> .
Ouaième	-	250 m	24 Sep. 1991	<i>Awaous guamensis</i> .
Oueiep	-	5 m	22 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. obscura</i> , <i>Kuhlia marginata</i> , <i>K. rupestris</i> , <i>Awaous guamensis</i> , <i>Cestraeus oxyrhynchus</i> , <i>C. plicatilis</i> .

Appendix 1. - (Continued).

Rivers	Stn	Altitude	Date	Species
Ouenghi	1	5 m	27 Jul. 1996	<i>Anguilla reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i>
	2	20 m	29 Jun. 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia marginata</i> , <i>K. rupestris</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> .
	3	60 m	29 Jun. 1996	<i>Anguilla australis schmidtii</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> .
Oue Thaboua	-	5 m	23 Sep. 1991	<i>Anguilla marmorata</i> , <i>Kuhlia marginata</i> , <i>K. rupestris</i> .
Padyéém	-	200 m	09 Oct. 1996	<i>Anguilla marmorata</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> , <i>S. sarasini</i> .
Pouébo	-	30 m	22 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia marginata</i> , <i>K. rupestris</i> , <i>Awaous guamensis</i> .
Pouembout	1	10 m	29 May 1996	<i>Anguilla australis schmidtii</i> , <i>A. marmorata</i> , <i>A. reinhardtii</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> .
	2	40 m	29 May 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Awaous guamensis</i> .
	3	100 m	06 Oct. 1996	<i>Anguilla reinhardtii</i> , <i>Awaous guamensis</i> .
	4	110 m	05 Oct. 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> , <i>S. sarasini</i> .
	5	490 m	28 May 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> .
Rivière blanche	-	180 m	03 Oct. 1991	<i>Anguilla megastoma</i> , <i>Galaxias neocaledonicus</i> .
Rivière bleue	-	180 m	05 Sep. 1991	<i>Galaxias neocaledonicus</i> .
Rivière des Pirogues	1	5 m	02 Oct. 1991	<i>Anguilla australis schmidtii</i> , <i>A. obscura</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> .
	2	180 m	11 Jun 1997	<i>Anguilla marmorata</i> , <i>A. megastoma</i> .
Rivière des lacs	-	245 m	17 Sep. 1991	<i>Galaxias neocaledonicus</i> .
Rivière du mois de mai	-	180 m	05 Sep. 1991	<i>Anguilla marmorata</i> , <i>Galaxias neocaledonicus</i> .
Tamoia	1	50m	09 Sep. 1991	<i>Anguilla reinhardtii</i> .
	2	400 m	14 Jun. 1997	<i>Anguilla australis schmidtii</i> , <i>A. marmorata</i> , <i>A. megastoma</i> , <i>A. reinhardtii</i> .
Tchamba	1	20 m	31 May 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Awaous guamensis</i> .
	2	70 m	08 Oct. 1996	<i>Anguilla marmorata</i> , <i>A. obscura</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> , <i>Sicyopterus taeniurus</i> .
Thio	3	40 m	08 Oct. 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Awaous guamensis</i> .
	1	35m	28 Jul. 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia marginata</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> .
	2	50 m	27 Jul. 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Sicyopterus taeniurus</i> .
Tite	3	140 m	27 Jul. 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Kuhlia rupestris</i> .
	-	80 m	23 Sep. 1991	<i>Anguilla marmorata</i> , <i>A. megastoma</i> , <i>Kuhlia marginata</i> , <i>K. rupestris</i> , <i>Awaous guamensis</i> .
Tiwaka	1	0 m	01 Jun. 1996	<i>Anguilla marmorata</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> , <i>Sicyopterus taeniurus</i> .
	2	40 m	30 May 1996	<i>Anguilla marmorata</i> , <i>Kuhlia marginata</i> , <i>K. rupestris</i> , <i>Awaous guamensis</i> .
	3	170 m	06 Oct. 1996	<i>Anguilla marmorata</i> , <i>A. reinhardtii</i> , <i>Sicyopterus taeniurus</i> .
	4	300 m	01 Jun. 1996	<i>Anguilla reinhardtii</i> .
Voh	-	0 m	20 Sep. 1991	<i>Anguilla marmorata</i> , <i>Kuhlia rupestris</i> , <i>Awaous guamensis</i> .



## COMMENTS ON THE FRESHWATER FISHFAUNA OF FRENCH POLYNESIA

by

Gérard MARQUET (1), Nathalie MARY (1) & Ronald WATSON (2)

**ABSTRACT.** - The compilation of the data of past and recent surveys allowed to record 37 species of freshwater fish in French Polynesia. The inventory is given and completed with remarks on some fish presenting biogeographical or economical interests. As a result, 10 species of gobies are endemic, belonging to the genera: *Lentipes*, *Sicyopterus*, *Sicyopus*, *Stenogobius* and *Stiphodon*. The juveniles of two species of *Sicyopterus* are commercially exploited and marketed as "ina'a".

**RÉSUMÉ.** - Remarques sur l'inventaire des poissons d'eau douce de Polynésie.

La compilation des données des récoltes anciennes et actuelles a permis de recenser 37 espèces de poissons dans les eaux douces de Polynésie française. La liste de ces espèces est complétée par des commentaires sur certaines d'entre elles qui présentent un intérêt biogéographique ou économique. Ainsi, dix espèces de gobies sont endémiques de Polynésie; elles appartiennent aux genres *Lentipes*, *Sicyopterus*, *Sicyopus*, *Stenogobius* et *Stiphodon*. Les juvéniles de deux espèces de gobies du genre *Sicyopterus* sont exploités et commercialisés sous le nom de "ina'a".

**Key-words.** - Gobiidae, French Polynesia, Ichthyofauna, Freshwater, Endemic species.

French Polynesia covers a vast oceanic region located near the eastern limit of the Indo-Pacific Province (Fig. 1), comprising 136 islands, with 35 highly volcanic and 83 low coral islands or atolls. The islands have a total area of 4,000 km<sup>2</sup>, scattered over 2.5x10<sup>5</sup> km<sup>2</sup> of ocean, and consist of five archipelagoes dispersed along a general north-west-southeast axis, i.e., Austral Is., Gambier Is., Marquesas Is., Society Is. and the Tuamotu Archipelago.

The freshwater fishes of French Polynesia have been neglected for a long time. Fowler (1932) gives a preliminary inventory for the Marquesas and Society Is. More regular surveys and studies started with Marquet (1988), followed by Watson (1991), Marquet and Galzin (1992), Maugé *et al.* (1992) and Watson (1995a, 1995b). Until recently, 32 species of fish were known from French Polynesia.

Recent surveys allowed to increase the inventory which is herein presented, and remarks on some species of gobies are given due to their biogeographical or economical interest.

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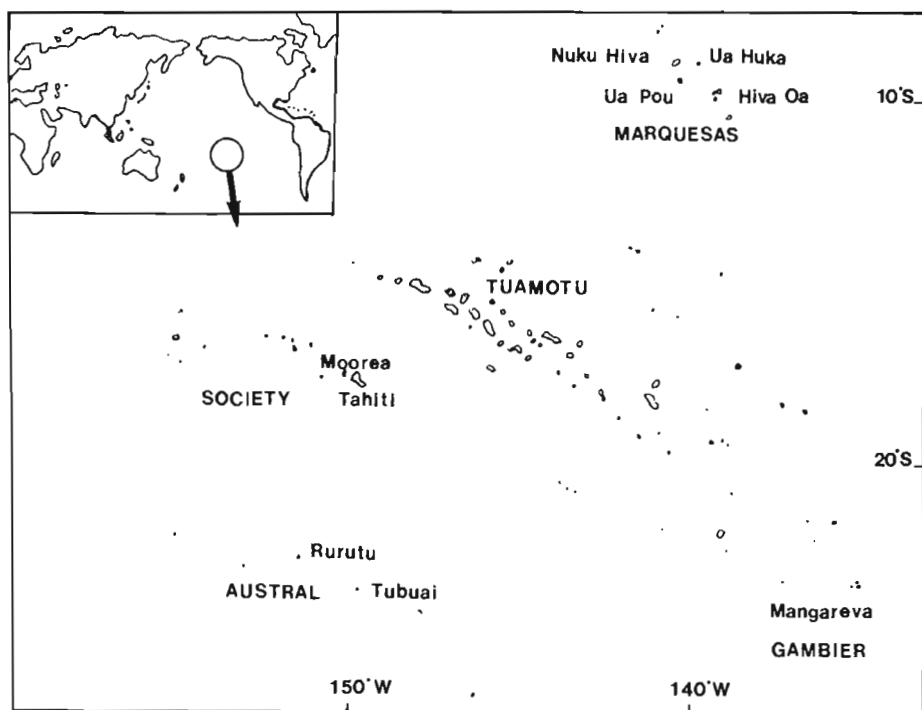


Fig. 1. - General map of French Polynesia showing the position of the nine relevant islands.

## MATERIALS AND METHODS

For the surveys, nine islands (Fig. 1) were chosen because of their accessibility and available logistic support provided by the Department of Rural Economy. These are Rurutu and Tubuai in the Austral Is., Mangareva in the Gambier Is., Hiva-Oa, Nuku-Hiva, Ua-Huka and Ua Pou in the Marquesas Is., Moorea and Tahiti in the Society Is.

The fish were mainly collected by electrofishing equipment, and occasionally rotenone was used.

## RESULTS

### Inventory

Table I gives the list of the 37 species of freshwater fish recorded in French Polynesia. The gobies are the most diverse with 14 species of 6 genera.

There are 10 endemic species belonging to the family Gobiidae: *Lentipes rubrofasciatus* Maugé et al., 1992, *Sicyopterus marquesensis* Fowler, 1932 (= *S. caudimaculatus* Maugé et al., 1992, according to Watson, pers. comm.), *Sicyopus bitaeniatus* Maugé et al., 1992, *Stenogobius caudimaculosus* Watson, 1991, *S. genivittatus* (Valenciennes, 1837), *S. marqueti* Watson, 1991, *S. randalli* Watson, 1991, *S. squamosus* Watson, 1991, *Stiphodon discotorquatus* Watson, 1995 and *S. tuivi* Watson, 1995. Table I gives the Polynesian localities for these endemic species.



### Geographical distribution

*Awaous ocellaris* (Broussonet, 1782), originally described from Tahiti, is now known from the Society and Austral archipelagoes westward to the Solomon Islands (Watson, 1992). *Stenogobius genivittatus* (Valenciennes, 1837), also originally described from Tahiti, is now considered as an endemic species of the Society Archipelago (Watson, 1991). *Stiphodon elegans*, described from Society Is. by Steindachner in 1880,

Table I. - Species of freshwater fishes recorded in French Polynesia with their distribution. A: Austral Islands; E: Endemic species; G: Gambier Islands; M: Marquesas Islands; S: Society Islands.

Families	Species	Islands
Anguillidae	<i>Anguilla marmorata</i>	A, G, M, S
	<i>A. megastoma</i>	A: Rurutu, G, S
	<i>A. obscura</i>	A, G, S
Moringuidae	<i>Moringua</i> sp.	S
Ophichthyidae	<i>Lamnostoma orientalis</i>	S: Tahiti
	<i>L. polyophtalma</i>	M: Nuku-Hiva
Poeciliidae	<i>Poecilia reticulata</i>	A: Tubuai, S
	<i>P. mexicana</i>	S: Tahiti
Syngnathidae	<i>Microphis brachyurus</i>	S
	<i>M. argulus</i>	M
Kuhliidae	<i>Kuhlia marginata</i>	A, S
	<i>K. mugil</i>	A: Tubuai, S: Tahiti
Carangidae	<i>Caranx melampygus</i>	A, S
	<i>C. papuensis</i>	M
	<i>C. sexfasciatus</i>	A, S
Lutjanidae	<i>Lutjanus fulvus</i>	A: Rurutu, S: Moorea
Cichlidae	<i>Oreochromis mossambica</i>	A: Tubuai, G, S
Mugilidae	<i>Chaenomugil leuciscus</i>	A: Tubuai
	<i>Liza alata</i>	M
	<i>Mugil cephalus</i>	A, G, S
	<i>Valamugil engeli</i>	A: Rurutu, S
Eleotrididae	<i>Eleotris fusca</i>	A, G, M, S
Gobiidae	<i>Awaous ocellaris</i>	A, S
	<i>Lentipes rubrofasciatus</i>	M: Ua-Huka (E)
	<i>Sicyopterus marquesensis</i>	M (E)
	<i>S. pugnans</i>	S
	<i>S. taeniurus</i>	A, G, M: Ua-Pou, Hiva-Oa, S
	<i>Sicyopus bitaeniatus</i>	M: Hiva-Oa, Ua-Pou (E)
	<i>Stenogobius caudimaculosus</i>	M: Nuku-Hiva, Ua-Huka (E)
	<i>S. genivittatus</i>	S: Moorea, Tahiti (E)
	<i>S. marqueti</i>	M: Hiva-Oa (E)
	<i>S. randalli</i>	A: Tubuai (E)
	<i>S. squamosus</i>	M: Ua-Pou (E)
	<i>Stiphodon discotorquatus</i>	A: Rurutu, Tubuai (E)
	<i>S. elegans</i>	A: Rurutu, S
	<i>S. tuivi</i>	M (E)
Siganidae	<i>Siganus spinus</i>	S

is a wide ranging species in the Central Pacific that is currently known from the Austral and Society archipelagoes in French Polynesia and the Samoa islands (Watson, 1995a). *Anguilla megastoma* described from Mangereva by Kaup (1856), is known from the Solomon islands to Pitcairn Is. (Ege, 1939) and from Rurutu and Gambier Archipelago (Marquet and Galzin, 1991).

### "Ina'a"

Juvenile stages of *Sicyopterus taeniurus* and *S. pugnans* are termed "ina'a" in French Polynesia and are considered a delicacy in this part of the world. These species are fished in river mouths and have to support a strong fishing effort. Ricard (1986) estimates that 3,500 kg of "ina'a" are taken annually, but Plessis (1976) evaluates the catch to be between 5 and 10 thousand kg per year. "Ina'a" are usually served as fritters.

## CONCLUSION

Some freshwater fishes of the French Polynesian have economic and/or biogeographic interest. Preservation measures should be taken to limit the many negative impacts they are subjected to, namely the growing urbanisation of valleys, the massive pig farming and the building of new dams.

**Acknowledgements.** - Sincere thanks are due to Walter Ivanstsoff (Macquarie University) for his meaningful comments and to Bernard Séret (ORSTOM) for his help in preparing the manuscript.

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## SHORT-TERM MOVEMENTS OF FRESHWATER FISHES IN SABAH, MALAYSIA

by

Keith MARTIN-SMITH (1, 2), Luke BULLOUGH (1) & Lindsay LAIRD (1)

**ABSTRACT.** - The short-term and short-distance movements of some freshwater fishes from Sabah, East Malaysia were determined by mark-release-recapture experiments. Fishes were captured by multiple pass electro-fishing, batch marked with visible implant elastomer tags in a variety of head and body positions and released at the point of capture. Recaptures were made at the original marking site and at representative sites up to 250 m up- and down-stream, at intervals from four days to one month after tagging. Pool-dwelling cyprinids such as *Garra borneensis*, *Lobocheilos bo* and *Osteochilus chini* showed high rates of movement over short time scales; for example, marked fish were recaptured up to 180 m from the tag site within 10 days. In contrast, the riffle-dwelling balitorids *Gastromyzon danumensis* and *Homaloptera stephensoni* were highly site-specific with up to 80% of recaptures occurring in the original tag location. However *Gastromyzon lepidogaster* was far more mobile with a substantial proportion of fish recaptured at different locations. Most movements of balitorids were small (< 30 m) but some individuals moved up to 165 m within 17 days. All species showed significant differences in the direction of movement with the majority showing significantly greater upstream displacement. These movements may be explained in terms of relative stability of the habitat and swimming ability of the fishes.

**RÉSUMÉ.** - Mouvements à court terme de poissons d'eau douce à Sabah, Malaisie.

Les mouvements à court terme et à courte distance de quelques poissons d'eau douce de Sabah, Est Malaisie, ont été déterminés par la méthode de marquage et recapture. Les poissons ont été capturés par pêche électrique et marqués sur le site de capture avec des implants en élastomère dans différentes parties du corps et de la tête. Les recaptures ont été faites sur le site de marquage et 250 m en amont et aval, à des intervalles de quatre jours à un mois. Les Cyprinidae des cuvettes profondes comme *Garra borneensis*, *Lobocheilos bo* et *Osteochilus chini* ont montré des taux élevés de mouvements sur des périodes courtes. Par exemple, les poissons marqués ont été repris jusqu'à 180 m du site de capture original dans les 10 jours. En revanche, les Balitoridae des zones peu profondes à eaux vives, comme *Gastromyzon danumensis* et *Homaloptera stephensoni*, étaient très localisés: environ 80% des recaptures ont été faites sur le site original. Cependant, *Gastromyzon lepidogaster* est beaucoup plus mobile avec une proportion notable des poissons repris à des sites différents. La plupart des mouvements de Balitoridae ont été réduits (< 30 m) mais certains individus se sont déplacés de 165 m en 17 jours. Toutes les espèces ont montré des différences significatives dans la direction des mouvements, la plupart montrant nettement une préférence pour les déplacements en amont. Ces mouvements peuvent être expliqués par la stabilité relative de l'habitat et la capacité de nage des poissons.

**Key-words.** - Cyprinidae, Balitoridae, Malaysia, Segama River, Movement, Site-fidelity, Tagging.

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Community organisation in fishes is strongly influenced by the individual movement patterns of the component species. Species which occupy a restricted home range generally show strong interactions with other species, often displaying territoriality (Jenkins, 1969; Hughes, 1992). If removed or displaced from their home ranges, repopulation may take some time (Armstrong *et al.*, 1994). Conversely, "opportunistic" species that display little site fidelity and wide-ranging exploratory movements may colonise new habitats quickly (Peterson and Bayley, 1993; Sheldon and Meffe, 1995). Both types of behaviour may be manifest within the same species; the population consisting of a proportion of site-associated individuals along with a number of itinerants (Saunders and Gee, 1964; Kennedy, 1981).

Recovery from habitat disturbance will be strongly dependent on the patterns of movement of fish species (Armstrong *et al.*, 1994). Timber extraction (logging) is one anthropogenic activity that can affect physical, chemical and biological characteristics of streams and rivers (see review by Campbell and Doeg, 1989). Populations of fishes can be severely reduced or become locally extinct if habitat disturbance is severe (Graynoth, 1979; Garman and Moring, 1993). Re-establishment of fish communities will, to a large extent, depend upon movements of fishes from unaffected areas (Meffe and Sheldon, 1990; Sheldon and Meffe, 1995).

The present paper is part of a larger study investigating the effects of selective logging on tropical fish communities in Sabah, Malaysia (Martin-Smith, *in press*). This study addresses current concerns over the maintenance of biodiversity and sustainable management of tropical rainforests (Marsh, 1995; Kottelat and Whitten, 1996).

Methods for the quantification of fish movements include direct observation, mark-recapture and radio-telemetry (e.g., Moyle and Baltz, 1985; Freeman, 1995; Matheney and Rabeni, 1995). Direct observation is often preferable since it causes less disruption but can be difficult to implement. Radio-telemetry can give precise information about movement but is normally restricted to only a few individuals because of cost. Mark-recapture inevitably involves disturbance to the fish community with possible effects on mortality and behaviour patterns but is often the only feasible method. Mark-recapture was used in the current study because of stream turbidity, large numbers of individuals and the time span of the planned experiments.

The aim of this paper was to quantify the movements of some abundant species of fish in Sabah over the time scale of days-weeks and the spatial scale of tens to hundreds of metres. The temporal and spatial scales were constrained by the limitations of the marking technique and logistics of sampling.

## MATERIAL AND METHODS

This study was carried out in five streams in the vicinity of Danum Valley Field Centre, Sabah (4°57'N, 117°40'E). All these streams are in the catchment of the Segama River and are described and mapped in Martin-Smith (1998). Streams are heterogeneous with "pool-and-riffle" geomorphology. Fish assemblages in pools are dominated by Cyprinidae, whereas Balitoridae (hillstream loaches) are abundant and speciose in riffles (Martin-Smith, 1998). The species which were chosen for study were *Garra borneensis*, *Lobocheilos bo* and *Osteochilus chini* (Cyprinidae) and *Gastromyzon danumensis*, *G. lepidogaster* and *Homaloptera stephensoni* (Balitoridae). These species were chosen because of their high abundance, maximising the numbers of individuals which could be marked and thus increasing the possibility of recapture.

Fish were batch-marked with Visible Implant Elastomer (VIE) tags (Northwest Marine Technology, Shaw Island, Washington); a two-part polymer which is mixed, injected into unpigmented tissue and allowed to cure into a flexible solid. Initial studies were conducted on suitable body locations for implanting tags, the effects of handling and tagging and tag loss over time (Bullough 1996; Martin-Smith unpubl. data). The most suitable location for tagging cyprinids was in the pre-opercular groove although there was significant tag loss over time from this location, restricting the length of study to < 50 days (Bullough, 1996). For balitorids, tags were located in the ventral adipose tissue between the pectoral and pelvic fins for *Gastromyzon* species and on the ventral thoracic surface for *H. stephensoni*; tag loss was negligible from these locations (Martin-Smith, unpublished data). All species showed no significant mortality due to handling or tagging (Bullough, 1996).

Three mark-recapture experiments on pool species were conducted between 31 May and 15 July 1996 (Table I). Pools were selected after visual inspection for the presence of target species. Source pools were isolated at the top and bottom with stop nets (5 mm mesh) and fish were captured by multiple-pass electrofishing (3 or 4 passes). Fish were held in holding buckets, lightly anaesthetised in 0.12 g l<sup>-1</sup> benzocaine solution, tagged, measured and returned to an aerated recovery bucket. Following complete recovery of all tagged fishes (approx. 30 min) they were returned to the middle of the source pool and the stop nets removed. Fish were recaptured by single-pass electrofishing on three occasions for each experiment, between 4 and 25 days after tagging. The source pool was fished on two of the three recaptures; in addition sites 0-250 m up- and downstream were fished, further sites being fished at later sampling dates. The exact location and dates of recaptures were dependent on logistics and weather and therefore varied between experiments, but equal effort was apportioned to upstream and downstream recaptures. All captured fish of the study species were examined for the presence of a tag, measured and returned.

Two mark-recapture experiments were performed on riffle-dwelling balitorids between 21 September 1996 and 11 July 1997 (Table II). Capture and tagging were done in a similar manner as described for cyprinids above. In the first experiment fish were captured at three different tagging sites (T1, T2, T3) with a distance of 30 m between T1 and T2 and 165 m between T2 and T3. Fish were batch-marked with a different tag colour or position at each location. Recaptures were made at 4, 18, 32, 164 and 292 days after tagging at the original tag sites and at five other riffle locations (A1-5) variously located along the stream (Fig. 1). Only data for recaptures at 4, 18 and 32 days are reported here, since these time points are on a comparable time scale to the cyprinid mark-recapture experiments. In the second experiment fish were batch-marked at two locations 23 m apart and one recapture event undertaken at 28 days at the tag sites only.

Data from the cyprinid mark-recapture experiments were standardised to percentage recapture of estimated remaining tagged fish using retention data from Bullough (1996); data from the balitorid experiments were left unmanipulated. In order to undertake parametric analyses, recapture data were combined from all time points for each set of experiments to ensure large enough sample sizes, giving total number of tagged fish for each species ( $N_t$ ) and the total number of recaptures ( $N_r$ ). For each species, the number of individuals recaptured at the original location ("static" individuals -  $N_s$ ) was compared with the number moving ( $N_m$ ) using the  $\chi^2$  statistic with one degree of freedom. The number of individuals moving upstream ( $N_u$ ) was compared with the number of individuals moving downstream ( $N_d$ ) also using the  $\chi^2$  statistic with one degree of freedom. Recapture data for all the species of Balitoridae were combined because of the low value of  $N_m$ .

Table I. - Summary of experimental procedure and number of fish tagged for cyprinid mark-recapture experiments.

Dates	River	Number of fish tagged			Time (days)	Recapture events	
		<i>Garra borneensis</i>	<i>Lobocheilos bo</i>	<i>Osteochilus chini</i>		Max. distance upstream (m)	Max. distance downstream (m)
31 May - 23 Jun. 96	LON	52	69	283	4/5, 7, 24	220	130
15 Jun. - 02 Jul. 96	CSR	12	71	57	4/5, 10, 17	125	120
21 Jun. - 14 Jul. 96	BKT	130	90	0	5/6, 8, 25	100	115

Table II. - Summary of experimental procedure and number of fish tagged for balitorid mark-recapture experiments.

Dates	River	Site	Number of fish tagged			Recapture events
			<i>Gastromyzon danumensis</i>	<i>Gastromyzon lepidogaster</i>	<i>Homaloptera stephensoni</i>	Time (days)
22 Sep. 96- 12 Jul. 97	PTA	T1	51	46	17	4, 18, 32, 164, 292
		T2	27	43	8	
		T3	35	40	12	
13 Jun. 97- 11 Jul. 97	BIL	BRT1	62	4	9	28
		BRT2	70	5	4	

Table III. - Summary of recaptures for all species. See text for details of abbreviations. ns = not significant at  $p = 0.05$ ; \* = significant at  $0.05 > p > 0.01$ ; \*\*\* = significant at  $p < 0.001$ .

Species	Number of tagged and recapture fish		Comparison of static vs. moving fish			Comparison of upstream vs. downstream movement		
	Nt	Nr	Ns	Nm	$\chi^2$ (1 d.f.)	Nu	Nd	$\chi^2$ (1 d.f.)
<i>Garra borneensis</i>	194	39	22	17	1.60 <sup>ns</sup>	4	13	4.76*
<i>Lobocheilos bo</i>	230	86	59	27	29.77***	22	5	10.70***
<i>Osteochilus chini</i>	340	65	52	15	51.08***	12	3	5.40*
<i>Gastromyzon danumensis</i>	275	48	40	8	53.33***	7	1	12.57***
<i>Gastromyzon lepidogaster</i>	138	28	17	11	1.29 <sup>ns</sup>	11	0	(all species combined)
<i>Homaloptera stephensoni</i>	50	17	13	4	5.40*	2	2	(all species combined)

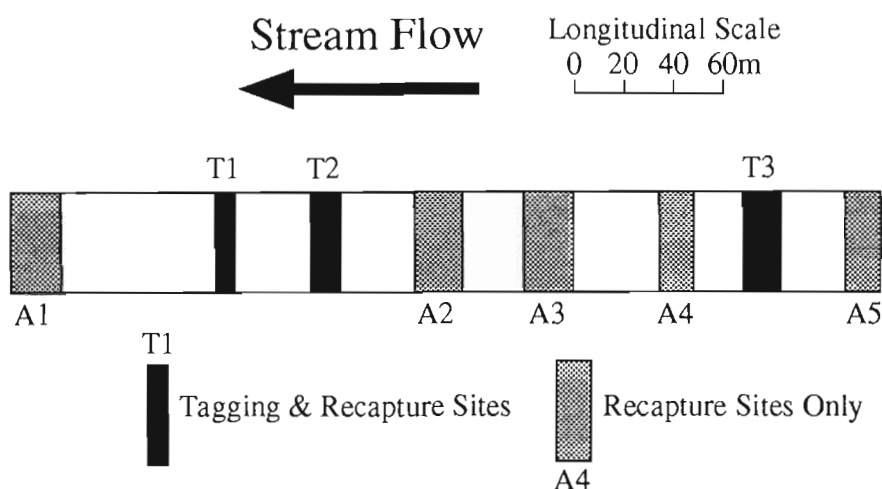


Fig. 1. - Schematic diagram of balitorid mark-recapture experiment at site PTA.

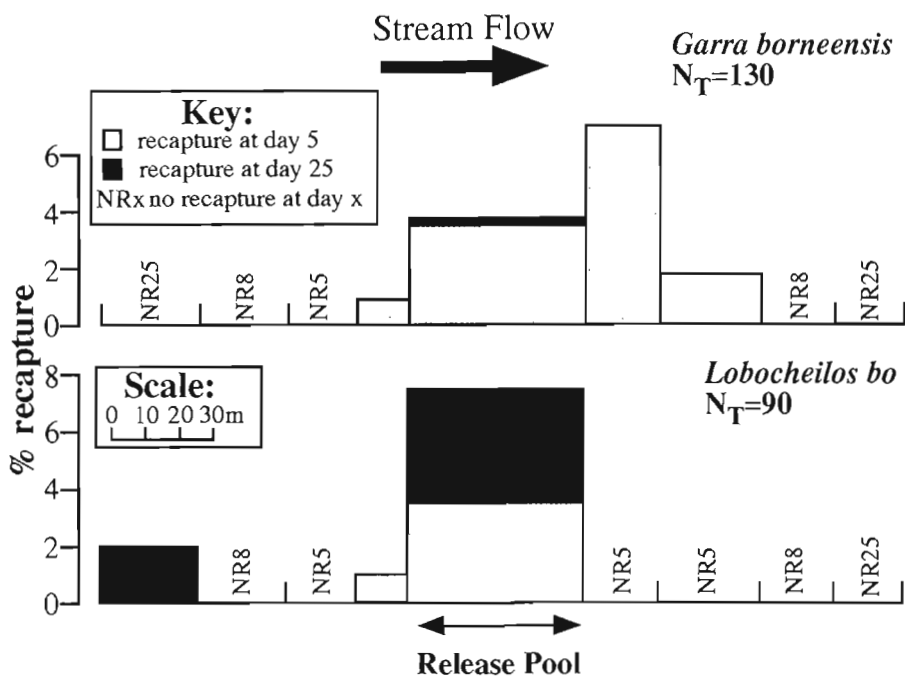


Fig. 2. - Distribution of cyprinid recaptures from site BKT. Note that recaptures at release pool are superimposed not sequential.

## RESULTS

All three species of cyprinid showed considerable movement during the experiments (Figs 2-4). The numbers of *Lobocheilos bo* tagged were fairly similar in each ex-

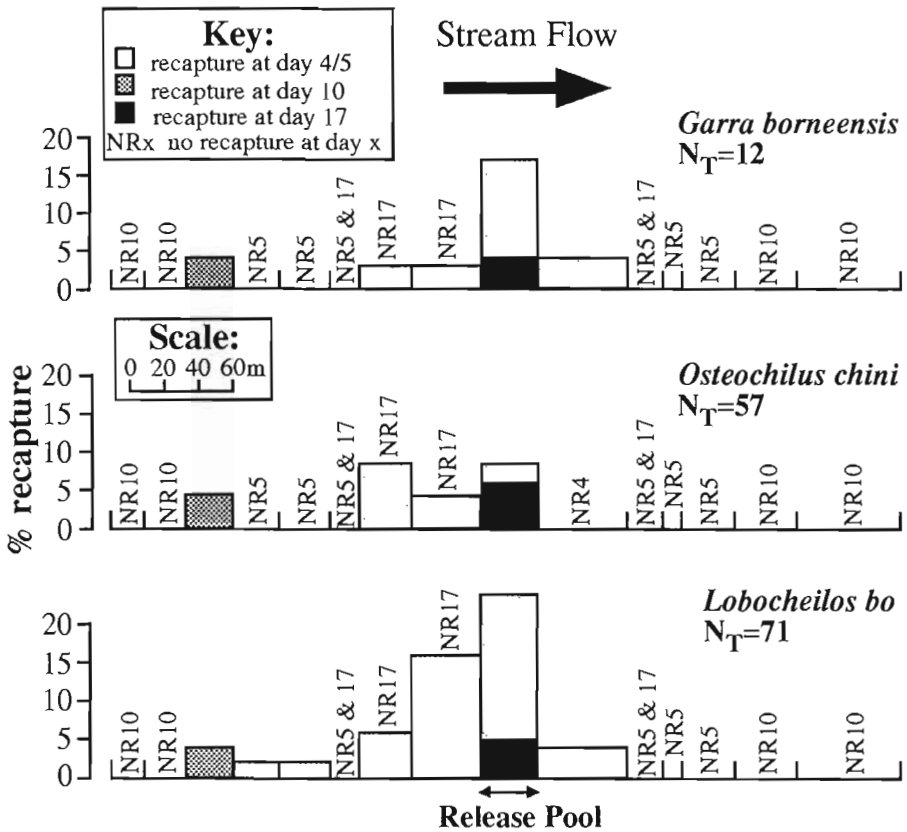


Fig. 3. - Distribution of cyprinid recaptures from site CSR. Note that recaptures at release pool are superimposed not sequential.

periment but numbers of *Garra borneensis* and *Osteochilus chini* varied considerably (Table I). Recaptures of all species were made at all time points, but absolute numbers of recaptures were quite small. *G. borneensis* was the most mobile of the cyprinids, with no significant difference between the number of static and moving individuals (Table III). There were significantly more downstream than upstream movements for *G. borneensis* (Table III). The maximum distance of recapture was 180 m upstream (day 10 at site CSR) and 90 m downstream (day 5 at site LON). *L. bo* showed the highest proportion of recaptures (37%) with significantly more static than moving individuals (Table III). Directionality of movement was also apparent with significantly more individuals captured upstream than downstream (Table III). The maximum distance of recapture was 180 m upstream (day 10 at site CSR) and 90 m downstream (day 5 at site LON). *O. chini* was the most site-associated of the cyprinids, with a highly significantly proportion of static individuals; moving individuals did so primarily in an upstream direction (Table III). The amplitude of movement was similar to the other cyprinids; maximum upstream movement 180 m (day 10 at site CSR), maximum downstream movement 60 m (day 5 at site LON). For all species and all experiments downstream recaptures were only made on the first sampling date, while upstream recaptures were made at later time points.



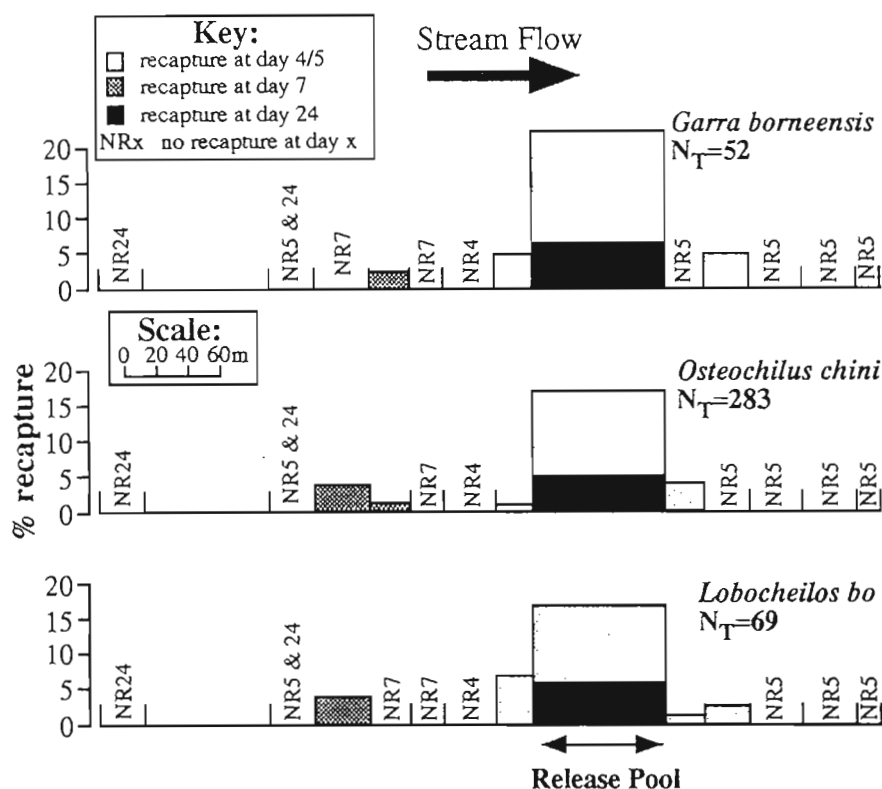


Fig. 4. - Distribution of cyprinid recapture from site LON. Note that recaptures at release pool are superimposed not sequential.

There were quite different recapture patterns between the different species of balitorids, especially the congeneric *Gastromyzon danumensis* and *G. lepidogaster* (Figs 5, 6; Table III). *Gastromyzon danumensis* was the most site-associated of all the balitorids, with few recaptures of moving fish. The number of static fish was significantly greater than the number of moving fish (Table III). In contrast, *G. lepidogaster* showed no significant difference between the number of static and moving fish (Table III). *Homaloptera stephensoni* appeared to be quite site-associated but unfortunately numbers of tagged fish were low. When data for all balitorids that moved were combined there were significantly more individuals that moved upstream than downstream (Table III). In experiment 1, there were no downstream movements, while there were upstream movements of *G. danumensis* of 30 m (day 18), *G. lepidogaster* up to 165 m (day 18) and *H. stephensoni* up to 90 m (day 32). The only downstream movements that were observed were in experiment 2, where one *G. danumensis* and two *H. stephensoni* moved 23 m downstream.

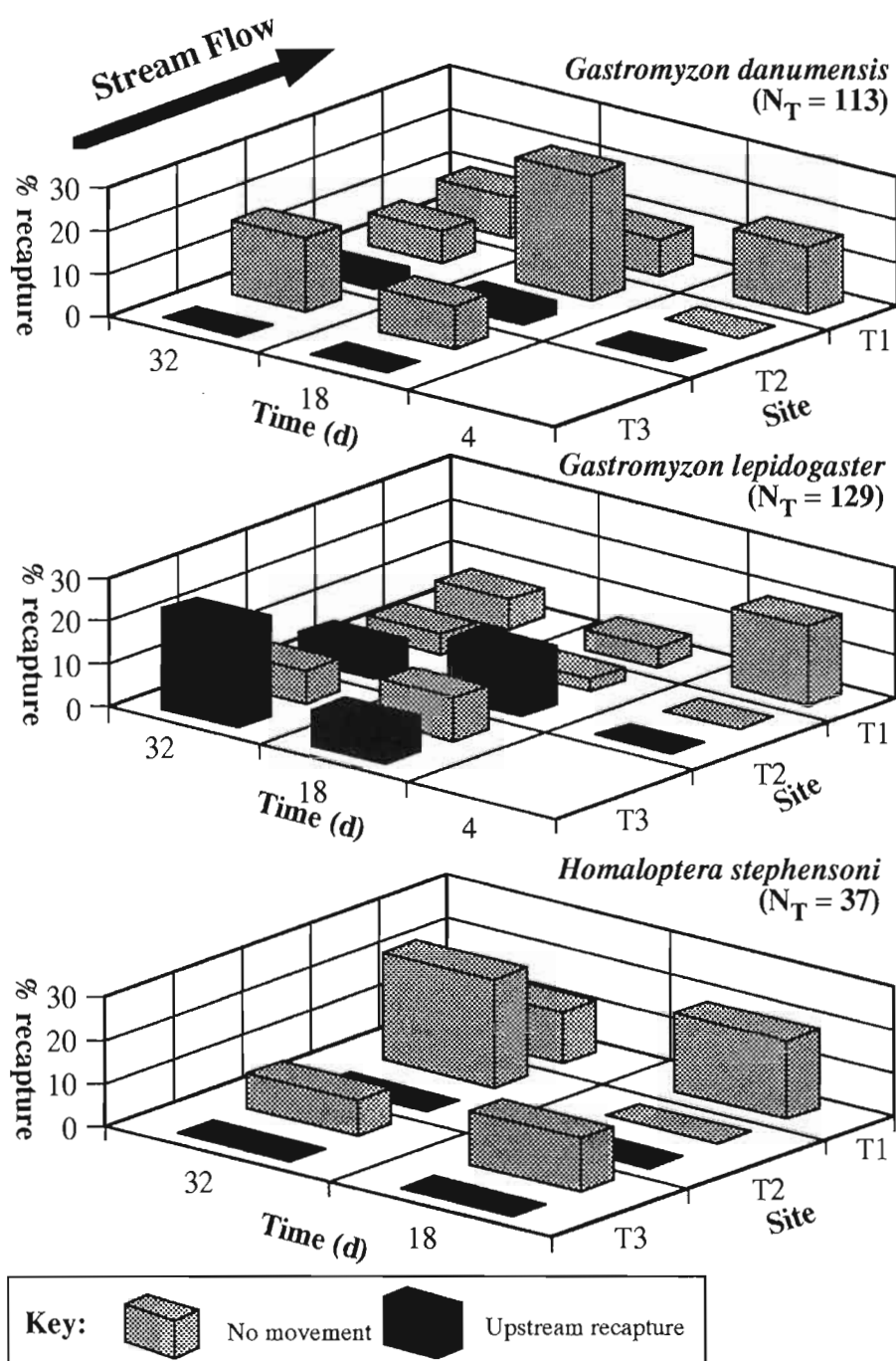


Fig. 5. - Distribution of balitorid recaptures from site PTA. Additional recaptures of 2 *Gastromyzon lepidogaster* and 2 *Homaloptera stephensoni* from sites A2 and A3 at day 32 not shown (see Fig. 1).

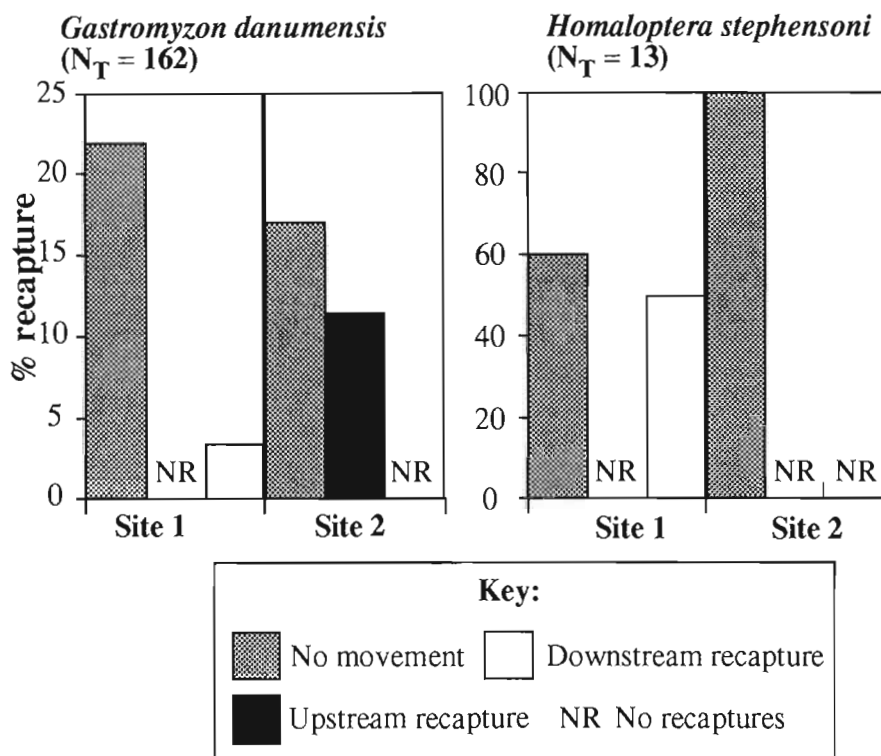


Fig. 6. - Distribution of balitorid recaptures from site BIL.

## DISCUSSION

The fishes in this study showed substantial differences in their mobility over a spatial scale of tens-hundreds of metres and a temporal scale of days-weeks. In general, cyprinids were more mobile than balitorids, although there were static and moving individuals in all species. For those individuals that moved, there was a greater tendency towards upstream movement, especially among the balitorids. However, it must be emphasised that the experimental design used is heavily biased towards the detection of static individuals and those moving relatively short distances (Gowan and Fausch, 1996). Fish making rapid, long-distance movements are generally missed and this may give a misleading view of a population consisting of individuals with small, restricted home ranges. Over 80% of cyprinids were not recaptured; all of these fishes could have moved out of the study area (i.e., > 250 m in either direction). Longer distance movements can only be quantified by sampling at more remote locations and/or the use of fish traps but both options are logistically demanding and fish traps are ineffective with highly variable water levels as are found in Sabah streams (Martin-Smith, in press).

Other potential artifacts include over-estimation of static fish through multiple recapture and alteration of behaviour patterns by capture and tagging disturbance (Gatz *et al.*, 1987). Finally, recaptures can provide information about endpoints only- intervening

movements are unrecorded. Thus the data from this study cannot be necessarily extrapolated to the whole population but do indicate that at least a proportion of all the species studied showed restricted or short-distance movement. Given the paucity of information on movements in tropical freshwater fish even an incomplete data set such as this is valuable.

Whether fish are considered to be mobile or static depends to a large extent on the definition of the spatial scale used- for example Funk (1957) considered fish that moved less than 1.6 km as "static" while Hill and Grossman (1987) used 50 m as the minimum criterion for movement. Freeman (1995) recaptured two species in a stream over 18 months and found the majority of recaptures within 33 m of the original capture location: she considered movements > 33 m to be "long-distance". In the current study fishes were marked within a recognisable, distinct geomorphological unit (i.e., a particular pool or a riffle). Since most of the studied species showed distinct habitat preferences (Martin-Smith, 1998), recapture in another location meant that an individual had crossed a different habitat area of low preference. This represents a directed behavioural response sufficient to be considered a distinct movement.

Little is known about the movement of tropical freshwater fishes (Yap and Furtado, 1980) but similar patterns of movement have been found in temperate species- for example, populations of juvenile trout consisted of static and mobile components with mobile individuals moving mostly in an upstream direction (Bridcut and Giller, 1995), while two thirds of recaptures of five species of Centrarchidae in Tennessee were less than 100 m from the previous capture site (Gatz and Adams, 1994). Freeman (1995) recaptured the majority of two stream fishes within 33 m of the original capture location; longer distance movements occurred in both upstream and downstream directions.

A range of movement patterns has been shown for temperate cyprinids, from restricted home ranges for yellowfin shiners (Goforth and Foltz, 1998) to high mobility for a number of large river species (Linfield, 1985) or a mixture of static and moving individuals in the minnow (Kennedy, 1981). A mixture of static and moving individuals may represent the outcome of different strategies of optimal foraging and patch exploration (Stephens and Krebs, 1986). *G. borneensis* and smaller *L. bo* are generally seen in large schools which may explain high proportions of moving individuals, since single pools may not provide enough resources to support a large school of fish for an extended period. However, *O. chini* is also found in large schools yet appears to be more site associated. Further work on *O. chini* in a larger river has confirmed the static nature of most individuals of this species (Lewis and Martin-Smith, unpubl. data). Yap and Furtado (1980) found that a tagged population of *Osteochilus hasselti* in peninsular Malaysia showed limited movements (average distance 15 m) and that most movements were in an upstream direction.

This study is the first reported investigation of the movements of balitorids. These fish have been assumed to be sedentary and site-associated due to their specialised body morphology (Inger and Chin, 1962), but this assumption has not been explicitly tested. Although *G. danumensis* and *H. stephensoni* showed a high proportion of static individuals, *G. lepidogaster* showed equal numbers of moving and static fish. All movements that were observed were upstream and some were of considerable distance (165 m in 18 days). *Gastromyzon lepidogaster* attains a considerably greater size (up to 105 mm SL) than *G. danumensis* (40 mm SL) which may explain the difference between the species. Further work involving tagging of > 1200 individuals of three *Gastromyzon* spp. has confirmed

that most individuals (> 90%) remain in the same riffle for periods up to two months (Lewis and Martin-Smith, unpubl. data).

The significance of upstream versus downstream movement is unclear. Rapid changes in water level are common in the streams of Danum Valley Field Centre which may displace fish downstream. Directed upstream movements may compensate for such displacement. However, balitorids are adapted to adhere to rock surfaces in high flow conditions and have been recaptured in the original locations immediately following flood events (Martin-Smith *et al.*, unpubl. data). Directed upstream movements in balitorid species may be an evolutionary strategy to fully exploit and colonise hillstreams, especially those with erratic flow regimes.

Given that the distances moved by fishes in this study are minimum movements, the potential for recovery of communities following disturbance is high, if there is free movement of fish from undisturbed areas. Cyprinids should be the first species to recolonise given their greater proportion of moving individuals and that they moved both upstream and downstream. Recolonisation of adult balitorids will occur primarily from upstream movement; thus upstream barriers could potentially affect balitorid colonisation dynamics.

**Acknowledgements.** - We would like to acknowledge Yayasan Sabah (Forestry Upstream Division), Danum Valley Management Committee, State Internal Affairs and Research of Sabah Chief Minister's Department, Sabah Fisheries Department and Economic Planning Unit of the Prime Minister's Department for permission to conduct research in Sabah. Our collaborators, the Sabah Fisheries Department and Abdullah Samat of Universiti Kebangsaan Malaysia have been most helpful. The help of Adrian Marshall, the field assistance of Johnny Salleh bin Otong and Karim Kasil, the criticisms of anonymous reviewers are gratefully acknowledged. This work has been funded by ODA-NERC, The Royal Society, Small Project Grant 1228B from the British Ecological Society, and Grant No. 95/31/13 from the 100% Fund of Fauna and Flora International. This is part of Danum Valley Research and Training Programme project no. 105 and publication no. A/208 of the Royal Society Southeast Asian Rainforest Research Programme.

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## ESTUARINE FISHES OF THE THA-CHIN RIVER, SAMUT SAKORN, THAILAND

by

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**ABSTRACT.** - A survey of the estuarine fishes of the Tha-Chin River in the Gulf of Thailand near Bangkok was carried out in May and July 1997 using a bamboo beam net (6.5 m wide, 16.0 m long and 1.5 cm codend). Abiotic factors including depth, temperature, salinity, pH and distance from shoreline were recorded. As a result, 55 species of fish were obtained from 7 stations (both day and night collections), representing 39 genera and 26 families. Most of the fishes were relatively small and immature. The most abundant species were *Ambassis gymnocephala* (Ambassidae) in number and *Eleutheronema tetradactylum* (Polynemidae) in biomass. Slight differences were observed in fish diversity between night and day collections. Several species are economically important, e.g., *Stolephorus commersonii* and *Mugil subviridis*. The recent development of human activities (pollution, urbanization, shrimp farming) caused the reduction of the mangrove areas and consequently that of the fish populations.

**RÉSUMÉ.** - Les poissons estuariens de la rivière Tha-Chin, Samut Sakorn, Thaïlande.

Un inventaire écologique des poissons estuariens de la rivière Tha-Chin, se jetant près de Bangkok dans le Golfe de Thaïlande, a été effectué en mai et juillet 1997, en utilisant un chalut à perche en bambou (6,5 m d'ouverture, 16,0 m de long et 1,5 cm de maille de cul). Les paramètres physico-chimiques suivants ont été mesurés: la profondeur, la température, la salinité, le pH et la distance à la côte. Au total, 55 espèces de poissons ont été récoltées dans 7 stations, de jour comme de nuit; elles représentent 39 genres et 26 familles. La plupart des poissons récoltés étaient petits et immatures. L'espèce la plus abondante en nombre était *Ambassis gymnocephala* (Ambassidae), et *Eleutheronema tetradactylum* (Polynemidae) la plus abondante en biomasse. De légères différences ont été observées entre les récoltes de jour et de nuit. Plusieurs espèces sont commercialement importantes comme *Stolephorus commersonii* et *Mugil subviridis*. Le développement récent des activités humaines (pollutions, urbanisation, fermes aquacoles à crevettes) dans l'estuaire a provoqué la réduction de la mangrove et par conséquent celle des populations de poissons.

**Key-words.** - Estuarine fishes, ISEW, Thailand, Mangrove, Monitoring.

Estuaries are known to be among the most complicated ecosystems. Physical and chemical parameters such as salinity are not stable, organisms living there must be adapted to survive in such changing habitat. In the tropics, banks of estuaries often have

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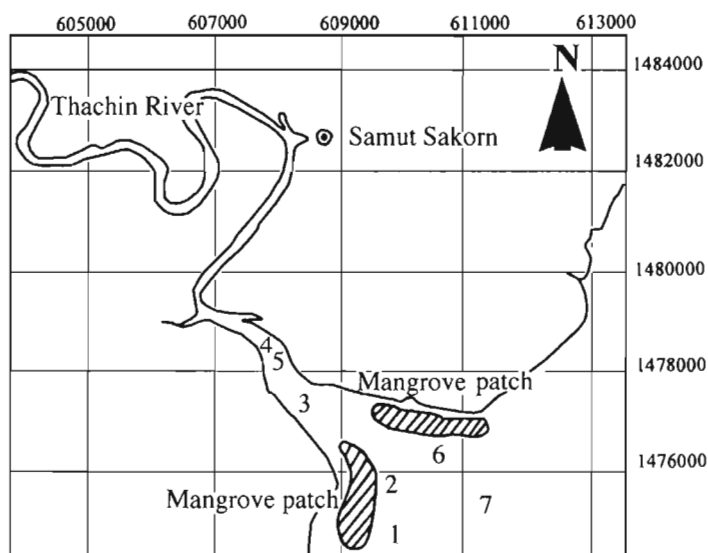


Fig. 1. - Aerial view of the estuary of the Tha-Chin river (Samut Sakorn, Thailand) showing the 7 stations sampled.

mangroves that serve as nursery grounds for many aquatic organisms including economically important fish and prawn species. In Thailand, especially on shoreline around the Gulf of Thailand, there were extensive areas of mangrove forests which are now severely diminished by invasion of human communities, shrimp farming and other impacts.

The Tha-Chin river, a side channel of the Chaophraya River, reaches the sea at Samut Sakorn province. Along its 325 km length, the Tha-Chin passes several large towns including Supanburi, Nakorn Pathom and Samut Sakorn. Many pollutants are discharged from sewage, sugar mills and other industries in the river so its water quality is poor. As a result of these human activities, the mangrove areas are rapidly decreasing.

As part of a general program of reforestation, a survey of the fish fauna was undertaken in the Tha-Chin river estuary. The objectives of this survey were: 1) to inventory the fish species of the estuary, 2) to establish baselines to monitor fish resources in polluted and destroyed mangrove area, and 3) to gain basic knowledge for further research such as building databases and fishery management, in estuarine habitats of Thailand.

## MATERIALS AND METHODS

The survey took place in May and in July 1997 in the estuary of the Tha-Chin river and consisted of 7 stations: 6 within the remaining patches of mangrove and one in the middle of the bay (Fig. 1). Fishes were collected with a bamboo beam net (6.5 m wide, 16.0 m long and 1.5 cm codend) towed at 1 knot by a 33 HP engine boat of 8.5 m long. Day hauls lasted 10 min and 5 min by night. In the field, the fishes were sorted by family, counted and weighted. Samples were preserved in 10% formaldehyde solution and deposited at the laboratory of zoology, Kasetsart University, for species identification.

The water quality parameters at each sample sites are given in table I.



## RESULTS

In the Tha-Chin estuary, 55 species were collected, representing 39 genera (27 were recorded by Wattachai, 1979) and 26 families (Table II). Herrings (Clupeidae) are the most diversified with 7 species, followed by the croakers (Sciaenidae) with 5 species and the carangids with 4 species. Some of freshwater fishes such as the snakehead (*Channa striata*) and the swamp eel (*Fluta alba*) accidentally occurred during day time. Mudskippers (*Periophthalmus* spp.) have been observed but could not be collected with the gears used during the survey. Migratory fishes occurred in the estuary such as some carangids which are found near shore during daytime but off shore at night. Some freshwater fishes, such as *Channa striata*, *Fluta alba* and *Anabas testudineus*, enter the estuary during flood at day time when the salinity is around 4-7‰.

Tables III and IV give the numbers and the weights of the fishes collected in May and July 1997 respectively.

In May, 3857 fishes were collected for a total weight of 11,298 g (Table III). Fishes of the following families: Polynemidae, Mugilidae, Scatophagidae and Engraulidae, represented about 60% of the biomass. In number of fishes, the Clupeidae were the most numerous, then the Ambassidae, Leiognathidae and Polynemidae; the fishes of these 4 families represented about 62% of the total catch.

In July, 8659 fishes were collected for a total weight of 16,717 g (Table IV). Fishes of the following families: Ambassidae, Polynemidae, Leiognathidae and Clupeidae represented 72% of the biomass. In number, fishes of these families represented 80% of the total catch.

Polynemids are among the most important fishes in the estuary, since they are abundant in biomass and in number during the two months of the survey, with the most common species: *Eleutheronema tetradactylum*. Ambassids, namely *Ambassis gymnocephalus*, represents also an important component of the fish fauna in the estuary.

## DISCUSSION

The fish diversity of Tha-Chin river observed during our survey is somewhat lower than that reported by Dolar *et al.* (1991), Leh and Sasekumar (1991), Martosewojo and Soedibjo (1991), Janekarn (1993), Monkolprasit (1994) and UNDP/UNESCO (1991).

Table I. - Average of some water quality parameters for the 7 field stations in the Tha-Chin river, in May and July 1997.

Station	Parameters							
	pH		Salinity (ppt.)		Temperature (°C)		Depth (m)	
	May	July	May	July	May	July	May	July
1	8.0	8.5	16.4	24.8	32.3	28.4	1.00	1.30
2	7.2	8.4	7.4	22.9	33.1	29.3	2.00	1.40
3	7.6	8.3	6.7	15.7	33.5	30.4	1.00	1.40
4	7.5	7.7	4.6	15.8	33.4	30.5	0.85	1.25
5	7.5	8	4	14.8	33.5	30.8	1.20	0.90
6	7.6	8.1	5.7	16.2	34.4	30.3	1.20	1.00
7	7.8	8.3	9.1	18.2	32.5	29.9	4.75	3.10

This may result of pollutions and overfishing in the river. However, the dominant groups are the same as those reported in southern Thailand (Monkolprasit, 1983) and in Malaysia (Chong *et al.*, 1991). Catfishes (Ariidae and Bagridae) are rather rare, compared

Table II. - List of fishes found in the estuary of the Tha-Chin river in May and July 1997.

Family	Scientific name	May	July
Clupeidae	<i>Anodontosoma chacunda</i>	-	+
	<i>Clupea karnagurta</i>	-	+
	<i>Clupeoides lile</i>	+	+
	<i>Clupeoides hypselosoma</i>	-	+
	<i>Corica laciniata</i>	+	+
	<i>Harengula dispilonotus</i>	+	+
	<i>Sardinella melanurus</i>	+	-
Engraulidae	<i>Stolephorus commersonii</i>	+	+
	<i>Stolephorus tri</i>	-	+
Ariidae	<i>Arius sagor</i>	-	+
Plotosidae	<i>Plotosus canius</i>	+	-
Belonidae	<i>Tylosurus anulatus</i>	+	-
Hemirhamphidae	<i>Hemirhamphus gaimardi</i>	+	+
	<i>Hemirhamphus marginatus</i>	+	-
Mugilidae	<i>Mugil dussumieri</i>	-	+
	<i>Mugil oligolepis</i>	-	+
	<i>Mugil subviridis</i>	+	+
	<i>Mugil troscheli</i>	+	+
	<i>Mugil borneensis</i>	+	+
Atherinidae	<i>Atherina valenciennei</i>	-	+
Polynemidae	<i>Eleutheronema tetradactylum</i>	+	+
Channidae	<i>Channa striata</i>	+	-
Flutiidae	<i>Fluta alba</i>	+	-
Symbranchidae	<i>Macrotrema caligans</i>	-	+
Abassidae	<i>Ambassis gymnocephala</i>	+	+
Theraponidae	<i>Therapon jarbua</i>	+	+
Sillaginidae	<i>Sillago sihama</i>	+	+
Carangidae	<i>Atule mate</i>	-	+
	<i>Selar kalla</i>	+	+
	<i>Chorinemus lysan</i>	-	+
Leiognathidae	<i>Chorinemus tala</i>	+	+
	<i>Leiognathus brevirostris</i>	+	+
	<i>Secutor insidiator</i>	+	+
Gerreidae	<i>Gerres oyena</i>	+	-
	<i>Gerres abbreviatus</i>	+	+
Sciaenidae	<i>Johnius novaehollandiae</i>	-	+
	<i>Johnius jabutus</i>	+	+
	<i>Johnius melanobranchium</i>	+	+
	<i>Johnius dussumieri</i>	+	+
	<i>Otolithoides argenteus</i>	+	+
	<i>Pana peramata</i>	+	-
	<i>Sciaena dussumieri</i>	+	+
Drepanidae	<i>Drepane punctata</i>	+	+
Scatophagidae	<i>Scatophagus argus</i>	+	+
Anabantidae	<i>Anabas testudineus</i>	+	-
Eleotridae	<i>Prionobutis koiomatodon</i>	+	+
Gobiidae	<i>Ctenogobius cylindriceps</i>	+	+
	<i>Ctenogobius criniger</i>	+	+
	<i>Glossogobius giuris</i>	+	+
	<i>Macgregorella moroana</i>	+	+
	<i>Trypauchen vagina</i>	+	+
Periophthalmidae	<i>Scartelaos viridis</i>	+	+
Cynoglossidae	<i>Cynoglossus puncticeps</i>	-	+
	<i>Cynoglossus monopus</i>	+	+
	<i>Cynoglossus oligolepis</i>	+	-

Table III. - Numbers (n°) and wet weight (W, in grams) of the fishes collected in the Tha-Chin river in May 1997 (D = day time, N = night time).

Station	1D		1N		2D		2N		3D		3N		4D		4N		5D		5N		6D		6N		7D		7N		Total weight (grams)	Total number
Family	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°		
Polynemidae	276	50	16	2	346	46	47	9	640	183	65	23	210	72	0	0	60	18	232	32	338	64	124	18	102	10	95	6	2551	533
Mugilidae	286	48	36	2	437	34	13	2	135	69	104	13	122	41	1	2	239	95	220	25	71	37	147	17	246	26	0	0	2057	411
Scatophagidae	84	1	45	2	711	26	0	0	54	2	0	0	0	0	0	0	32	1	0	0	0	0	0	0	132	7	62	4	1120	43
Engraulidae	108	42	20	6	152	42	15	3	177	73	35	2	143	44	0	0	73	20	26	5	235	62	21	5	18	4	6	1	1029	309
Clupeidae	61	70	63	61	87	54	36	51	154	105	23	29	136	124	3	6	103	62	77	84	46	30	39	24	14	15	33	14	875	729
Ambassidae	86	63	26	17	127	95	28	15	24	19	20	17	15	14	70	23	67	55	95	65	67	54	53	42	73	51	54	35	805	565
Sciaenidae	25	7	57	59	19	6	19	21	18	1	32	40	42	5	17	63	18	2	39	7	1	1	58	10	117	28	87	19	549	269
Leiognathidae	54	111	15	33	9	11	8	16	9	10	32	66	17	9	10	41	55	30	23	64	10	4	14	20	41	68	97	76	394	559
Gobiidae	13	3	4	2	0	0	7	5	26	4	7	3	64	5	1	1	5	1	64	8	0	0	35	5	9	4	134	24	369	65
Carangidae	92	38	1	1	59	12	0	0	0	0	0	0	0	0	0	0	8	2	0	0	44	10	0	0	92	23	40	9	336	95
Gerreidae	0	0	6	3	0	0	4	1	0	0	6	7	0	0	4	6	48	19	32	36	11	11	10	11	3	1	112	28	236	123
Channidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	226	1	0	0	0	0	0	0	0	0	0	0	226	1
Pteropthalmyidae	0	0	13	3	0	0	47	11	0	0	40	9	21	5	31	8	24	7	6	2	0	0	0	0	0	0	0	0	182	45
Hemirhamphidae	7	3	0	0	28	8	0	0	41	8	0	0	35	13	0	0	22	6	0	0	18	5	0	0	0	0	0	0	151	43
Cynoglossidae	0	0	0	0	0	0	63	1	0	0	47	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	1	122	3
Anabantidae	0	0	0	0	0	0	0	0	102	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	102	1
Belontiidae	0	0	1	1	0	0	8	4	0	0	17	3	0	0	0	0	0	0	33	7	0	0	4	1	0	0	3	1	66	17
Theraponidae	0	0	0	0	0	0	0	0	5	2	19	3	8	6	1	1	3	1	3	1	5	2	0	0	0	0	10	2	54	18
Sillaginidae	0	0	0	0	0	0	0	0	4	2	2	2	6	2	14	3	10	5	0	0	4	5	0	0	0	0	0	0	40	19
Drepanidae	2	1	0.8	2	0	0	0	0	0	0	0.5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.3	4
Synbranchidae	0	0	0	0	0	0	0	0	0	0	0	0	25	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	1
Plotosidae	0	0	0	0	0	0	0.2	1	3	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.2	3
Ariidae	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1

Table IV. - Numbers (n°) and wet weight (W, in grams) of the fishes collected in the Tha-Chin river in July 1997 (D = day time, N = night time).

Station	1D		1N		2D		2N		3D		3N		4D		4N		5D		5N		6D		6N		7D		7N		Total weight (grams)	Total number
Family	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°	W	n°		
Ambassidae	2730	1412	375	201	30	16	106	63	47	31	10	5	80	69	20	11	390	28	520	348	110	98	380	259	17	9	70	25	4885	2575
Clupeidae	100	68	90	30	15	16	15	6	701	815	5	1	706	853	5	2	40	46	5	2	10	8	10	1	3	1	95	51	1800	1900
Leiognathidae	240	71	25	13	35	8	10	11	20	5	5	2	1	1	0	0	30	26	845	479	5	2	1065	573	110	44	165	104	2556	1339
Polynemidae	65	8	20	1	60	11	0	0	610	193	0	0	1890	863	5	1	70	23	20	4	20	3	35	7	25	3	15	1	2835	1118
Gerreidae	10	6	0	0	10	10	0	0	40	40	20	13	30	28	20	21	40	78	290	291	65	114	275	264	0	0	5	1	805	866
Engraulidae	412	109	10	1	70	17	0	0	245	67	0	0	80	19	0	0	10	2	0	0	0	0	0	0	0	0	55	15	882	230
Mugilidae	150	32	15	6	78	20	17	6	90	50	15	8	245	74	10	1	50	6	155	4	30	6	100	5	7	1	7	1	969	220
Sciaenidae	3	2	10	2	20	1	30	16	10	3	15	18	0	0	5	7	1	1	0	0	0	0	30	5	5	1	115	70	244	126
Theraponidae	20	2	0	0	355	69	0	0	22	4	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0	402	76
Gobiidae	7	1	40	10	83	14	10	4	1	1	25	4	40	5	35	8	20	5	25	2	10	2	15	1	0	0	10	5	321	62
Sillaginidae	0	0	0	20	13	5	2	27	25	0	0	0	0	1	1	5	3	0	0	0	0	0	0	0	0	0	0	0	58	44
Periophthalmidae	0	0	50	9	0	0	100	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	150	29
Carangidae	25	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1	0	0	0	0	10	2	20	5	60	16	
Hemirhamphidae	24	6	10	1	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	8
Cynoglossidae	0	0	15	2	0	0	15	3	10	2	50	3	0	0	10	4	1	1	0	0	0	0	0	0	0	0	0	0	101	15
Scatophagidae	250	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	230	2	0	0	70	2	0	0	550	14	
Atherinidae	5	4	0	0	0	0	0	0	2	3	0	0	0	0	0	0	1	2	0	0	1	2	0	0	0	0	10	2	19	13
Belonidae	0	0	0	0	0	0	10	1	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	4
Drepanidae	0	0	0	0	10	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	2
Eleotridae	0	0	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1
Synbranchidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	1	0	0	0	0	10	1	

to previous investigations by Monkolprasit (1994); this decline probably results from pollution as catfishes, being bottom dwellers, are sensitive to pollution.

Most of the fishes collected are small, and a number of them are at juvenile stage, such as *Scatophagus argus*, *Sillago sihama* and *Drepane punctata*, for which mangroves are nursery grounds (Bell *et al.*, 1984).

Human activities contribute to the reduction of fishes populations in the Tha-Chin estuary, namely the urbanization. The growth and settlements of fisherman villages, resorts and shrimp farms scattered around river mouth cause the destruction of mangrove areas every year, consequently the nursery grounds for many aquatic animals are regularly disappearing. Although a number of farms have been abandoned due to shrimp diseases, the mangrove has not recovered yet where these farms were situated.

Overfishing is another factor, as illegal fishing gears, such as purse seines and pushing nets, are currently used by fishermen. These gears are non-selective, and species with low commercial value, such as *Mugil* spp. and *Eleutheronema* are caught and used for animal meal.

Our preliminary survey of the Tha-Chin estuarine fish fauna should be completed and more quantitative data should be obtained to provide the necessary information for monitoring and management of the Tha-Chin mangrove and its biota.

**Acknowledgement.** - This project is part of funding research of Dr. Sanit Aksornkaew supported by the Thailand Research Fund. We would like to thank our colleagues from the Department of Marine Sciences, Chulalongkorn University, who kindly assisted us during the field survey, and those of the Kasetsart University (IPF4) particularly Prof. Supap Monkolprasit for providing a special grant to attend the 5th Indo-Pacific Fish Conference in Noumea.

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## DESCRIPTION OF A NEW SPECIES OF *ACANTOPSIS* (CYPRINIFORMES: COBITIDAE) FROM THAILAND

by

Suebsin SONTIRAT (1)

**ABSTRACT.** - The genus *Acantopsis* is so far known from Thailand by a single species, *A. choirorhynchos*, which exhibits various color patterns possibly due to ecological variations. The new species, *Acantopsis thiemmedhi*, is described from the Huey Mae Preeg (Lampang province), Huey Nam Khoon and Huey Song Thang, tributaries of Huey Thab Salao, Huey Kha Khaeng Wildlife Sanctuary (Uthai Thani province) and Chao Phraya river at Tambon Tha Nam Oi (Nakorn Sawan province). The new species differs from *A. choirorhynchos* by having larger spots on the lateral line, dark saddle-like patches along the back and a row of dark blotches or wavy lines in between, and one dark and large oblong patch on each caudal lobe. The number of gill rakers is 16-18 versus more than 20 in *A. choirorhynchos*.

**RÉSUMÉ.** - Une nouvelle espèce d'*Acantopsis* (Cypriniformes: Cobitidae) de Thaïlande.

Le genre *Acantopsis* ne comptait jusqu'à présent qu'une seule espèce en Thaïlande, *A. choirorhynchos*, laquelle présente de nombreux patrons de colorations, probablement dus à des adaptations écologiques. Nous décrivons une nouvelle espèce, *Acantopsis thiemmedhi*, provenant de Huey Mae Preeg (province de Lampang), Huey Nam Khoon et Huey Song Thang, affluents de Huey Thab Salao, de Huey Kha Khaeng (province de Uthai Thani) et de la rivière Chao Phraya à Tambon Tha Nam Oi (province de Nakorn Sawan). Cette espèce se distingue de *A. choirorhynchos* par sa coloration, des taches plus grandes au niveau de la ligne latérale, et foncées sur le dos, une rangée de lignes ondulées entre les deux, ainsi que de grandes taches foncées sur chaque lobe caudal. Le nombre de branchiospires varie de 16 à 18, contre plus de 20 chez *A. choirorhynchos*.

**Key-words.** - Cobitidae, *Acantopsis choirorhynchos*, *A. thiemmedhi*, Thailand, New species.

Although Smith (1945) mentions unique coloration for fishes of the genus *Acantopsis*, they have different color patterns which may be due to habitat variation and behavior (Fowler, 1935, 1937, 1939). So far, a single species of *Acantopsis* has been recorded from Thailand by Boulenger (1903): *A. choirorhynchos* (Bleeker, 1854) primarily described from Sumatra. A new species of *Acantopsis* from Thailand is herein described.

### MATERIALS AND METHODS

The materials examined are preserved in the collection of the Kasetsart University Museum of Fisheries (KUMF). The list of type specimens is given below. The 21 specimens collected by the author were compared to 180 specimens of *A. choirorhynchos*.

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Fig. 1. - Holotype of *Acantopsis thiemmedhi* n.sp., 117.6 mm SL (KUMF 3131) from Huey Nam Khoon, Uthai Thani province.

Counts and morphometric characters were taken according to Hubbs and Lagler (1964). Morphometrics were expressed as percentage of standard length (SL) and head length (HL).

### *ACANTOPSIS THIEMMEDHI* N. SP.

(Fig. 1, Table I)

*Acantopsis choirorhynchus*: Fowler, 1935: 35-42; 1937: 152; 1939: 59. Smith, 1945: 296-297.

#### Material examined

*Holotype*. - KUMF-3131, 11.76 cm SL, Huey Nam Khoon, Huey Kha Khaeng wildlife Sanctuary, Amphur Lan Sak, Uthai Thani province, captured by the author and team, at the altitude 200 m, on 10 Aug. 1996, by cyanide poisoning and net.

*Paratypes*. - 20 specimens. KUMF 3132 (16 spms), 4.67-11.46 cm SL, Huey Mae Preeg, Tambon Mae Preeg, Amphur Mae Preeg, Lampang province, captured by the author and team, at the altitude 250 m, on 10 Oct. 1996, by cyanide poisoning and net. KUMF 3133 (2 spms), 11.63-12.28 cm SL, Huey Song Thang, Huey Kha Khaeng Wildlife Sanctuary, Amphur Lan Sak, Uthai Thani province, captured by the author and team, at the altitude 160 m, on 11 Aug. 1996, by cyanide poisoning and net. KUMF 3134 (2 spms) 6.18-8.55 cm SL, Chao Phraya river at Tambon Tha Nam Oi, Amphur Payuhakiri, Nakorn Sawan province, captured by local fishermen, at the altitude of about 50 m, on 25 Sep. 1996, by net.

#### Diagnosis

A species of the genus *Acantopsis* with the following characters: 16-18 gill rakers; 8-9 dark saddle-like bars on back; 8-9 irregular dark blotches or wavy lines above the lateral line and 8-9 irregular and/or large dark round spots along the lateral line; a row of large oblong dark longitudinal spots near the rim of the dorsal fin and another dark spot



on the middle part of the first three front dorsal-fin rays; one large oblong dark bar on the middle part of each caudal lobe; a row of smaller vertical oblong dark spots in the middle part of the caudal fin and two or three dark spots on the caudal base; posterior pelvic rims dark.

### Description

Table I gives the main meristic and morphometric characters of the 21 specimens of the type series.

A small *Acantopsis* loach (maximum known size: 132 mm SL) with an elongate and slightly compressed body, covered with minute scales; origin of the dorsal fin in front of that of the pelvic fin, the latter being opposite to the 5th or 6th dorsal-fin ray (5th in holotype); origin of the pectoral fin in front of gill opening; caudal fin bilobed.

**Coloration.** - 8-9 (8 in holotype) dark saddle-like bars along the back; 8-9 (8 in holotype) irregular dark blotches or wavy lines above the lateral line; 8-9 (8 in holotype) irregular and large round dark spots along the lateral line; a row of oblong dark blotches near the dorsal-fin rim; a dark spot on the middle part of the first three dorsal-fin rays; one large oblong dark bar near the middle part of each caudal lobe; a row of small oblong dark

Table I. - Meristic and morphometric characters of *Acantopsis thiemmedhi* n. sp. and *Acantopsis choirorhynchos* expressed in percentage of standard length (SL) or head length (HL).

	<i>Acantopsis thiemmedhi</i> n. sp.			<i>Acantopsis choirorhynchos</i>	
	Holotype	21 paratypes		180 specimens	
		Range	Mean	Range	Mean
<b>Meristic characters</b>					
Dorsal-fin rays	ii+9	ii+9-10	ii+9	ii+9-10	ii+9.2
Pectoral-fin rays	i+12	i+10-12	i+12	i+9-10	i+9.4
Pelvic-fin rays	i+5	i+6	i+6	i+5-6	i+5
Anal-fin rays	ii+5	ii+5-6	ii+5	ii+6-7	ii+6.3
Gill rakers	16	16-18	16.5	21-27	22.8
Vertebrae	39	39-41	40	40-44	43
<b>Morphometric characters</b>					
Body depth (% SL)	13.5	12.3-15.2	13.5	9.8-13.0	11.8
Head depth (% SL)	53.5	53.0-59.5	58.0	39.1-46.8	45.0
Head length (% SL)	26.1	23.4-26.1	24.9	24.4-27.4	25.6
Eye (% SL)	14.1	13.7-16.6	14.9	11.0-15.3	13.6
Snout (% SL)	63.4	57.9-64.1	62.4	57.7-67.6	63.5
Postorbital length (% SL)	26.7	22.2-29.7	25.8	20.8-31.2	25.2
Interorbital length (% SL)	8.3	8.3-12.2	10.5	6.4-11.8	8.5
Predorsal length (% SL)	50.5	47.1-53.0	50.9	48.6-54.3	51.9
Prepectoral length (% SL)	27.8	20.4-27.8	22.4	21.9-25.2	23.5
Prepelvic length (% SL)	56.4	54.1-58.5	56.3	54.5-59.2	57.8
Preanal length (% SL)	82.7	78.8-83.4	79.9	72.2-86.6	81.2
Dorsal-fin base (% SL)	15.4	14.2-15.4	14.3	12.7-16.1	14.4
Anal-fin base (% SL)	10.5	5.5-10.5	7.9	6.0-9.6	8.1
Pectoral-fin length (% SL)	20.5	14.3-20.5	16.7	10.8-15.6	13.5
Pelvic-fin length (% SL)	14.5	8.5-14.5	9.5	7.6-11.1	9.3

spots along the middle part of caudal fin and two or three (3 in holotype) dark spots along the caudal-fin base; other fins hyaline.

### Distribution and ecology

*Acantopsis thiemmedhi* is known from the Mae Preeg stream of Lampang province, in the Huey Nam Khoon and Huey Song Thang; tributaries of Huey Tab Salao within the Huey Kha Khaeng Wildlife Sanctuary, Uthai Thani province and Chao Phraya river in Tambon Tha Nam Oi, Amphur Payuhakiri of Nakorn Sawan province. It is rarer than *A. choirorhynchus*, but both are found in mountain streams and the upper part of rivers with sandy bottoms where clear running water is present.

### Etymology

This new species is named in honor of Professor Jinda Thiemmedh, the former dean of the Faculty of Fisheries, Kasetsart University, who was my first teacher in the field of Ichthyology.

### Remarks

*Acantopsis thiemmedhi* differs from related species in having 16-18 gill rakers instead of more than 20 in other species of this genus. In addition, the color pattern is quite different, in that it has dark blotches or spots on caudal and dorsal fins, while *A. choirorhynchus* does not exhibit such markings. However, small dark lateral spots and/or blotches on the sides and back as well as three rows of small dark spots on the dorsal fin may or may not be present in small specimens of *A. choirorhynchus*.

The Thai name for *A. choirorhynchus* and other related species is «Pla Chon Sai» which refers to its habitat as well as its behavior, as it means fish that prefers to live and/or penetrate underneath the sand. This fish species also inhabits in between gravel and coarse sand.

**Acknowledgements.** - I would like to thank Mr. Aphinun Suwanaraksa for photographing the holotype specimen, Mr. Thongchai Champasri for drawing the figure and all my colleagues who helped in collecting specimens and preparing this manuscript.

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## GENERIC RELATIONSHIPS OF THE INTERNALLY-FERTILIZED SOUTHEAST ASIAN HALFBEAKS (HEMIRAMPHIDAE: ZENARCHOPTERINAE)

by

Amy Downing MEISNER (1) & Bruce B. COLLETTE (2)

**ABSTRACT.** - There is strong support for a monophyletic group, here recognized as the subfamily Zenarchopterinae Fowler, 1934, containing all five genera of internally-fertilized halfbeaks. Three genera, *Hemirhamphodon*, *Nomorhamphus*, and *Dermogenys*, are known to be internally fertilized and viviparous. *Zenarchopterus* (with about 20 species) and the monotypic genus *Tondanichthys* also appear to be internally fertilized but are apparently oviparous. A sister-group relationship between *Dermogenys* and *Nomorhamphus* is supported by modifications of the anal fin in males. Support is also strong for a sister-group relationship between *Hemirhamphodon* and (*Dermogenys* + *Nomorhamphus*). Monophyly of (*Dermogenys* + *Nomorhamphus*) is also well supported. *Dermogenys*, as currently constituted, appears to be paraphyletic. Within the group comprised of (*Dermogenys* + *Nomorhamphus*), two monophyletic groups are supported by new characters of sperm bundle morphology and modifications associated with viviparity. One clade comprises nine species of *Dermogenys*, including the type species, *D. pusilla*, and five undescribed species, and the other clade seven species of *Dermogenys* and four species of *Nomorhamphus*, including the type species, *N. celebensis*, and four undescribed species.

**RÉSUMÉ.** - Relations phylétiques des demi-becs (Hemiramphidae: Zenarchopterinae) à fécondation interne du Sud-Est Asiatique.

Plusieurs observations sont en faveur de la monophylie du groupe formé par les cinq genres de demi-becs à fécondation interne. Ils sont regroupés dans la sous-famille des Zenarchopterinae. Trois genres, *Hemirhamphodon*, *Nomorhamphus* et *Dermogenys* recourent à la fécondation interne et sont vivipares. *Zenarchopterus* (composé de près de 20 espèces) et le genre monotypique *Tondanichthys* sont aussi à fécondation interne, mais sont apparemment ovipares. La modification de la nageoire anale des mâles supporte l'hypothèse que *Dermogenys* et *Nomorhamphus* forment deux groupes frères. *Hemirhamphodon* est le groupe frère de (*Dermogenys* + *Nomorhamphus*). La monophylie de (*Dermogenys* + *Nomorhamphus*) est vraisemblable. *Dermogenys* tel qu'il est conçu semble être paraphylétique mais au sein du groupe (*Dermogenys* + *Nomorhamphus*) on observe deux groupes monophylétiques caractérisés par la morphologie des spermatozeugmes et les modifications associées à la viviparité. Un clade comprend neuf espèces de *Dermogenys*, y compris l'espèce-type, *D. pusilla*, et cinq espèces nouvelles. L'autre clade comprend sept espèces de *Dermogenys* et quatre espèces de *Nomorhamphus*, y compris l'espèce-type, *N. celebensis*, et quatre espèces nouvelles.

**Key-words.** - Hemiramphidae, Zenarchopterinae, Viviparous halfbeaks, Internal fertilization, Phylogeny.

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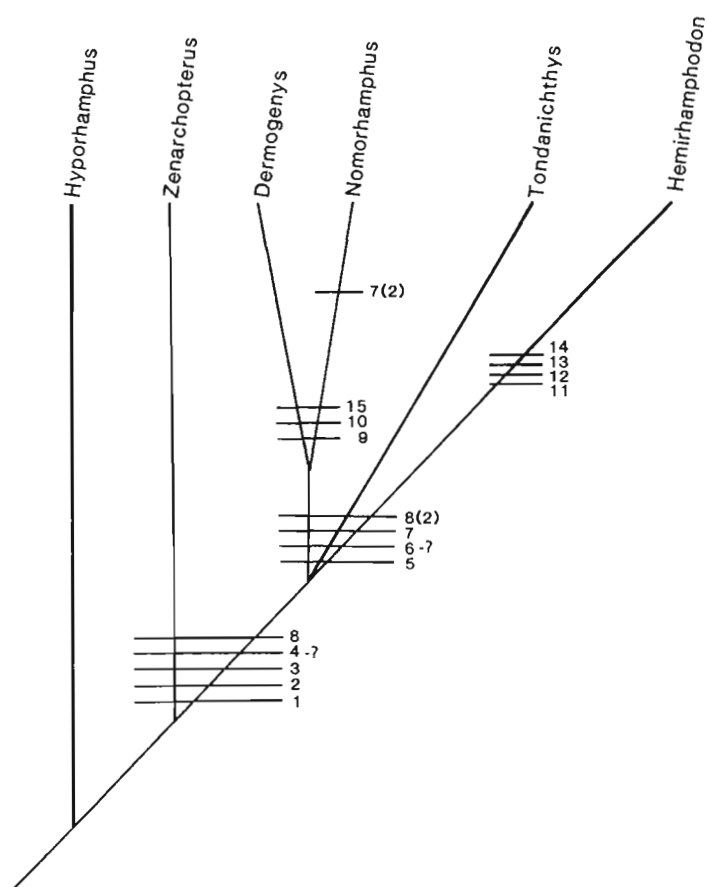


Fig. 1. - Previous hypothesis of relationships for the genera of internally-fertilized halfbeaks using *Hyporhamphus* as the outgroup. See Collette (1995) for characters.

The purpose of this paper is to outline relationships among the five genera of internally-fertilized halfbeaks herein defined as the subfamily Zenarchopterinae. The family Hemiramphidae contains 13 genera and more than 100 species of atherinomorph fishes commonly known as halfbeaks (Collette and Parin, 1998). Fowler (1934) described several new subfamilies of Hemiramphidae, including the Zenarchopterinae, composed of *Zenarchopterus*, *Hemirhamphodon*, *Arrhamphus*, and *Melapedalion*. *Arrhamphus* and *Melapedalion* are externally-fertilized, short-jawed monotypic genera of Hemiramphinae. Fowler also described the Dermogenyinae for *Dermogenys* and *Nomorhamphus*, but we consider these genera to be part of the Zenarchopterinae. Monophyly of the subfamily, as the *Zenarchopterus*-group, was hypothesized by Collette (1995) based on five synapomorphies: 1) some anal-fin rays modified in males; 2) reduced nasal fossa; 3) an elongate nasal barbel that projects well out of the nasal fossa; 4) reduced number of vertebrae (38-51); and, presumably 5) internal fertilization. Characters in spermatozoal ultrastructure were used by Jamieson and Grier (1993) to support monophyly of the group, which they recognized as the family Zenarchopteridae. Within the subfamily, three genera are inter-

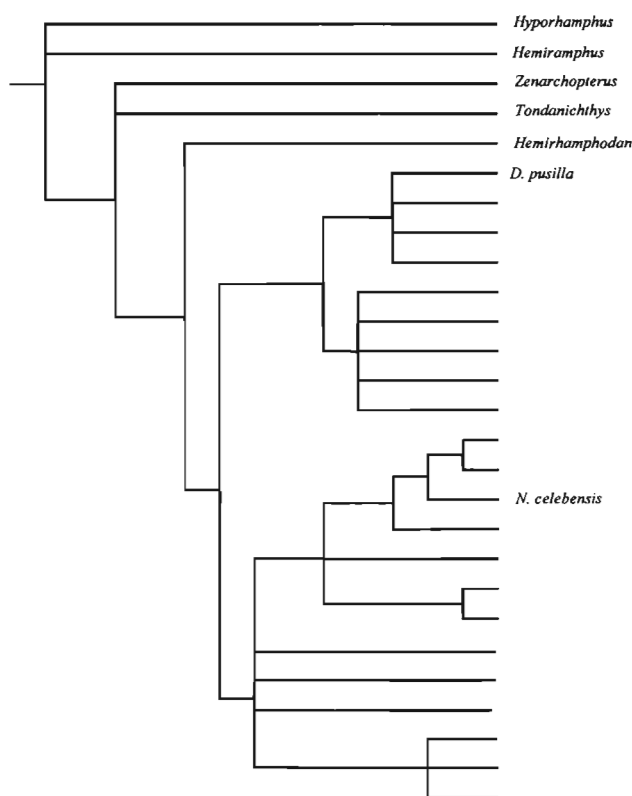


Fig. 2. - Revised hypothesis of relationships for the genera of Zenarchopterinae. Only the type species of *Dermogenys* and *Nomorhamphus* are labelled.

nally fertilized and viviparous: *Hemirhamphodon*, *Dermogenys*, and *Nomorhamphus*. Monophyly of *Hemirhamphodon* was supported in a revision by Anderson and Collette (1991) who also proposed a tentative phylogeny for the *Zenarchopterus* species group. Subsequently, Collette (1995) described the monotypic *Tondanichthys kottelati* and proposed a revised phylogenetic hypothesis (Fig. 1) for the viviparous genera using *Zenarchopterus* and *Hyporhamphus* as outgroups. *Tondanichthys* was described from immature individuals and was hypothesized to be viviparous based on this preliminary phylogeny.

In an effort to clarify relationships within the subfamily Zenarchopterinae, we integrate Collette's previous research on the three plesiomorphous genera with Meisner's (1998) research on *Dermogenys* and *Nomorhamphus*. Her research started when she was an undergraduate summer intern with the second author in 1992 studying a species group of *Dermogenys* and then expanded to include all the species placed in *Dermogenys* and *Nomorhamphus*. A systematic revision of *Dermogenys* and *Nomorhamphus* is in preparation by the first author.

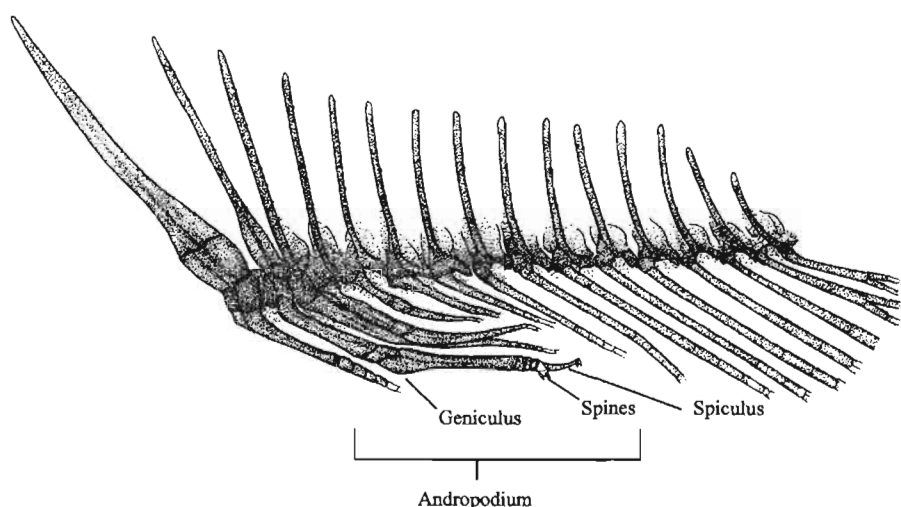


Fig. 3. - Diagrammatic representation of the modified anal-fin rays (andropodium) of *Dermogenys* sp. USNM 345500, 28.5 mm SL (from Meisner and Collette, in press). Middle radials absent and ossified; distal radials not illustrated; cartilage not illustrated; bone stippled. (Drawing by Keiko Hiratsuka Moore.)

## PHYLOGENY

When morphological and histological characters are combined to form an hypothesis of relationships (Fig. 2), *Tondanichthys* forms an unresolved trichotomy with *Zenarchopterus* and *Dermogenys* + *Nomorhamphus*, suggesting that it may not be viviparous. *Hemirhamphodon* is now placed as the sister-group to *Dermogenys* + *Nomorhamphus*. Monophyly of *Zenarchopterus*, *Tondanichthys*, and *Hemirhamphodon* is not controversial. However, species and generic definitions for two genera of southeast Asian fresh and brackish water species, *Dermogenys* and *Nomorhamphus* have been vague for many years. Part of the reason for this is that, in the past, no adequate defining characters had been proposed for either *Dermogenys* or *Nomorhamphus*. Consequently, if a new species in this group was discovered, it was described as a *Nomorhamphus* if it lacked an elongate lower jaw or as a *Dermogenys* if it had an elongate lower jaw. However, when all species are examined, it is clear that there is a continuum of jaw lengths making it difficult to define generic limits.

A character used by Collette (1995) to distinguish *Dermogenys* from *Nomorhamphus* was the supposed lack of true gill rakers on the first gill arch. This character is more widely distributed among the viviparous halfbeaks and is not diagnostic of either *Dermogenys* or *Nomorhamphus*. Further complicating the taxonomy of these genera is the fact that most species of both nominal genera have been described on the basis of overlapping meristic and morphometric characters (Brembach, 1991).

Histological characters suggest that within this last group, two clades are supported. One contains only nominal species of *Dermogenys* and is defined by the presence of large sperm bundles and intrafollicular development. The second clade contains nominal species of both *Dermogenys* and *Nomorhamphus* and is defined by small sperm bundles and a long period of intraluminal development (Downing and Burns, 1995; Meisner and Burns, 1997a).

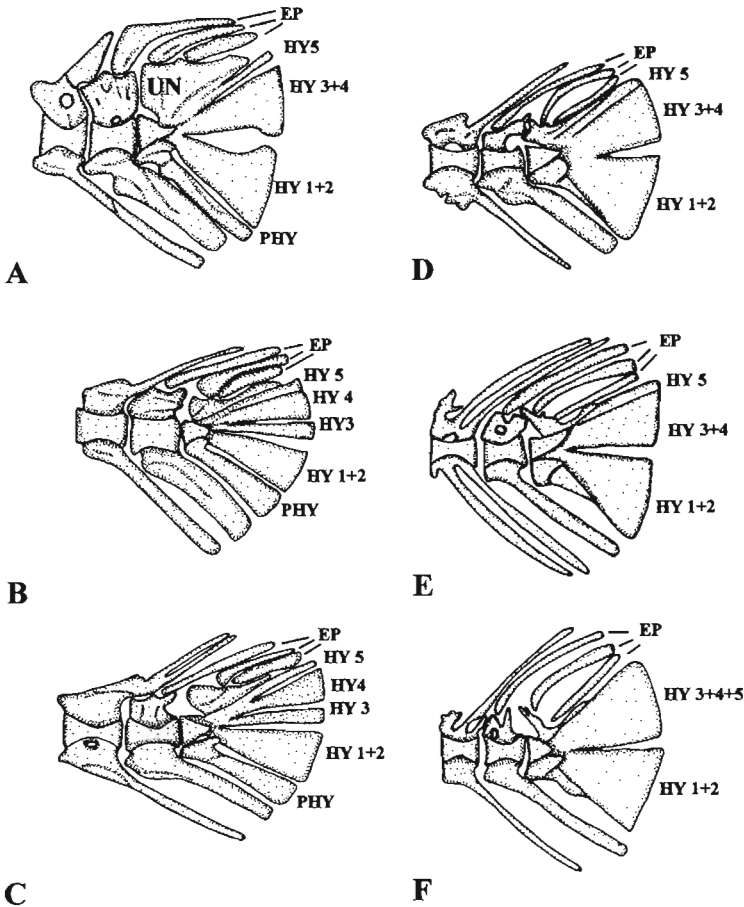


Fig. 4. - Diagrammatic representation of the caudal skeleton. EP, epurals; HY, hypurals; PHY, parhypural; UN, uroneural. **A:** *Hyporhamphus unifasciatus*, USNM 196819, 74.5 mm. **B:** *Zenarchopterus rasori*, USNM 263456 female, 64.0 mm. **C:** *Tondanichthys kottelati*, ZSM/CMK 7980 male, 61.5 mm. **D:** *Hemirhamphodon kuekenthali*, USNM 330828 male, 50.0 mm. **E:** *Nomorhamphus viviparus*, CAS 137829 male, 34.0 mm. **F:** *Nomorhamphus ebrardtii*, ZMH 7150 male, 50.0 mm. Cartilage not illustrated; bone is stippled.

Preliminary conclusions are that *Dermogenys* as currently constituted is paraphyletic. *Dermogenys* should be restricted to nine small species, including the type species, *D. pusilla*, and five undescribed species (Fig. 2). *Nomorhamphus* (type species *N. celebensis*), should be expanded to include the 13 larger species, some of which have been previously classified as *Dermogenys*, and four of which are undescribed.

One character supporting monophyly of the Zenarchopterinae is modification of the anal fin in males. Anal-fin modifications vary at both the generic and species levels. Some combination of anal-fin rays 5-7 are modified in *Zenarchopterus* (Collette, 1985). Brembach (1976, 1991) termed the modified anal fin of males of the viviparous genera an andropodium. The modified part of the anal fin in *Hemirhamphodon* is in the middle or

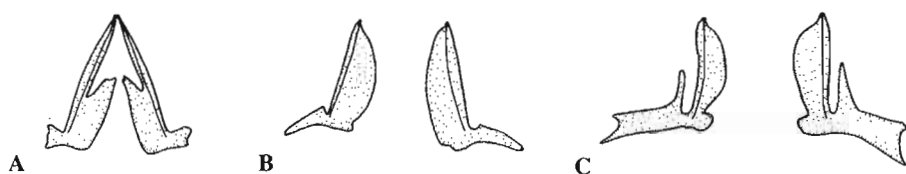


Fig. 5. - Ventral view of the right and left pelvic bones. A: representation of the condition observed in *Hyporhamphus*, *Hemiramphus*, *Tondanichthys*, and *Zenarchopterus* (*Hyporhamphus unifasciatus*, USNM 196819, 74.5 mm); B: *Hemirhamphodon kuekenthali*, USNM 330828 male, 50.0 mm; C: representation of the condition observed in *Dermogenys* and *Nomorhamphus* (*Nomorhamphus* sp. (Sulawesi) USNM 338490, female, 44.0 mm). Bone stippled; anterior towards the top.

posterior part of the fin (rays 4, 5, and 8 or just 5 and 8). The andropodium in species of *Dermogenys* (Fig. 3) and *Nomorhamphus* is essentially identical: the anterior 1-7 rays are thickened and curved posteriorly and the first anal-fin pterygiophore is enlarged. Previous revisions of *Dermogenys* and *Nomorhamphus*, utilized, to some extent, characters of the andropodium in defining species (Mohr, 1936; Brembach, 1976, 1991) but taxonomy of *Dermogenys* and *Nomorhamphus* has remained confused.

To understand ontogenetic changes in the anal fin of *Dermogenys* and *Nomorhamphus*, specimens of aquarium-raised *Dermogenys siamensis* were sacrificed at different stages of development (Meisner and Burns, 1997a). After reaching maturity, no further changes take place in the andropodium, so characters found in the andropodia of mature males can be used to differentiate species. Detailed descriptions of the anal fins and relationships among the species will be presented in the systematic revision by the first author.

Several characters unite the three viviparous genera, *Hemirhamphodon*, *Dermogenys*, and *Nomorhamphus*. These three genera share development of a wide cytoplasmic periaxonemal sheath on the sperm (Jamieson and Grier, 1993). Osteological characters involve a reduction in both the size and number of skeletal elements that may be correlated with an overall reduction in body size (Meisner, 1998).

For example, informative reductive characters are found in the caudal skeleton (Fig. 4). In *Hemiramphus* and *Hyporhamphus*, hypurals 1 + 2 are completely fused and are widely separated from completely fused hypurals 3 + 4. In *Zenarchopterus* and *Tondanichthys* (Fig. 4B-C), hypurals 3-5 are partially fused but free for most of their length. The bony mass of the caudal skeleton is reduced in *Hemirhamphodon*, *Dermogenys*, and *Nomorhamphus* (Fig. 4D-F); there are dorsal and ventral hypural plates, and the parhypural is fused to the ventral hypural plate.

A character that may be correlated with viviparity is found in the pelvic girdle. The viviparous genera have the pelvic bones widely separated (Fig. 5B, C) whereas the pelvic bones are close together in *Hemiramphus*, *Hyporhamphus*, *Zenarchopterus*, and *Tondanichthys* (Fig. 5A). In the viviparous genera, there is a lateral reorientation so that the pelvic bones lie along the anterior-posterior axis instead of having the anterior tips angled towards the midline.

Additional characters bearing on the phylogeny of the internally-fertilized half-beaks are present in the gonads (Grier and Collette, 1987; Downing and Burns, 1995; Meisner and Burns, 1997b) and sperm morphology (Jamieson and Grier, 1993). Monophyly of *Zenarchopterus* is supported by the presence of a bipartite testis structure, as well as the method of spermatzoegma formation (Grier and Collette, 1987). Monophyly of



*Dermogenys* + *Nomorhamphus* is supported by having only a single very elongate mitochondrion in the sperm (Jamieson and Grier, 1993), and the even distribution of spermatid nuclei around the periphery of the spermatocysts during spermatogenesis (Downing and Burns, 1995). Within that clade, two sperm bundle morphologies have been described, each suggesting a more inclusive group of species (Downing and Burns, 1995). Species of *Dermogenys* (*sensu stricto*) have large sperm bundles; species of *Nomorhamphus* have small sperm bundles.

Viviparity of the three most derived genera is one reason for their popularity as aquarium fishes. In some species superfetation, multiple broods developing simultaneously, is present. In *Dermogenys* and *Nomorhamphus*, five types of viviparity have been described, each unique to a more inclusive clade (Meisner and Burns, 1997b). Species of *Dermogenys* (*sensu stricto*) have intrafollicular development; species of *Nomorhamphus* have intraluminal development. The implications of these characters to the phylogeny of these genera will be included in the comprehensive revision of the genera by the first author.

## CONCLUSIONS

The relationships within the subfamily Zenarchopterinae, of five genera presumed to be internally fertilized, are clarified. Different types of characters are informative at different levels of analysis. Osteological characters are important in defining generic relationships within the Zenarchopterinae, clarifying the phylogenetic position of *Tondanichthys*, and showing that *Hemirhamphodon* is the sister-group of *Dermogenys* + *Nomorhamphus*. The taxonomy of the species of *Dermogenys* and *Nomorhamphus* has been in a state of confusion for over a century. Histological characters of the gonads provide the first unambiguous characters to separate *Dermogenys* from *Nomorhamphus*.

**Acknowledgements.** - We thank Mrs. Keiko Hiratsuka Moore for drawing figure 2. Drs. Andrew Cooper, Thomas Munroe, and Lynne Parenti and two anonymous reviewers all provided useful comments on drafts of the manuscript.

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## Symposium 2

### COASTAL FISHES OF REEFS, LAGOONS, AND MANGROVES

Chairperson: **Richard WINTERBOTTOM**

- GILL A.C. - Subspecies, geographic forms and widespread Indo-Pacific coral-reef fish species: A call for change in taxonomic practice. 79-87

*Catégorie taxinomique des poissons récifaux: espèces à large répartition géographique, sous-espèces ou formes géographiques? Invitation à un changement dans la pratique de la Taxinomie.*

- GLUCKMANN I., BUSSERS J.-C., POULICEK M. & P. VANDEWALLE. - Preliminary study of the morphology of the head in Pomacentridae adductor mandibulae organization in *Dascyllus aruanus* (Teleostei: Perciformes). 89-97

*Étude préliminaire de la morphologie de la tête des Pomacentridae: l'organisation de l'adductor mandibulae chez Dascyllus aruanus (Teleostei: Perciformes).*

- HUTCHINS J.B. - Biogeography of the nearshore marine fish fauna of the Kimberley, Western Australia. 99-108

*Biogéographie de la faune ichtyologique des côtes du Kimberley, Australie occidentale.*

- MEUNIER F.J. & L. ZYLBERBERG. - The structure of the outer components of the scales of *Latimeria chalumnae* (Sarcopterygii: Actinistia: Coelacanthidae) revisited using scanning and transmission electron microscopy. 109-116

*La structure des composants superficiels des écailles de Latimeria chalumnae (Sarcopterygii: Actinistia: Coelacanthidae) revue grâce au microscope électronique à balayage et à transmission.*

- MEUNIER F.J. & H. FRANCILLON-VIEILLOT. - Histological structure of the caudal spine of the surgeonfish *Ctenochaetus striatus* (Teleostei: Acanthuridae). 117-124

*Structure histologique des épines caudales chez le poisson-chirurgien Ctenochaetus striatus (Teleostei: Acanthuridae).*

- MEUNIER F.J., BÉAREZ P. & H. FRANCILLON-VIEILLOT. - Some morphological and histological aspects of hyperostosis in the Eastern Pacific marine fish *Prionotus stephanophrys* Lockington, 1880 (Triglidae). 125-133

*Quelques caractères morphologiques et histologiques de l'hyperostose chez un poisson marin du Pacifique Est, Prionotus stephanophrys Lockington, 1880 (Triglidae).*

- PARMENTIER É., CHARDON M., POULICEK M., BUSSERS J.-C. & P. VANDEWALLE. - Morphological particularities of the head in four Carapidae (Ophidiiformes) 135-146

*Particularités morphologiques de la tête chez quatre Carapidae (Ophidiiformes).*

## **SUBSPECIES, GEOGRAPHIC FORMS AND WIDESPREAD INDO-PACIFIC CORAL-REEF FISH SPECIES: A CALL FOR CHANGE IN TAXONOMIC PRACTICE**

by

Anthony C. GILL (1)

**ABSTRACT.** - Many apparently widespread Indo-Pacific coral-reef fish species can be divided into well-diagnosed, geographic forms, but these have been recognised as subspecies and geographic varieties. Criteria for recognising such forms at an intraspecific rather than specific level are shown to be unjustified. It is also argued that such allocation of geographic forms to intraspecific ranking implies phylogenetic and biogeographic relationships that may be unsupported by evidence, and may lead to unstable nomenclature. Moreover, because intraspecific forms are rarely distinguished in subsequent literature, information on their biology, morphology and distribution does not accumulate. Full specific status should therefore be awarded to well-diagnosed, geographic forms.

**RÉSUMÉ.** - Catégorie taxinomique des poissons récifaux: espèces à large répartition géographique, sous-espèces ou formes géographiques ? Invitation à un changement dans la pratique de la Taxinomie.

La diagnose de nombreuses espèces de poissons récifaux indo-pacifiques aboutit à leur subdivision en sous-espèces et variétés géographiques. Cette étude montre que les critères utilisés pour reconnaître ces formes au niveau intraspécifique plutôt qu'au niveau spécifique ne sont pas justifiés. Attribuer des rangs intraspécifiques à ces formes géographiques implique des hypothèses de relations phylogénétiques et biogéographiques sous-jacentes qui, éventuellement, ne sont pas prouvées, aboutissant ainsi à une nomenclature instable. De plus, les informations concernant la biologie, la morphologie et la répartition sont rarement rapportées à ces formes intraspécifiques dans la littérature. Un statut d'espèce devrait par conséquent être attribué aux formes géographiques dont la diagnose est suffisamment confortée.

**Key-words.** - Coral-reef fish, Geographic variation, Species, Subspecies, Taxonomy.

Indo-Pacific coral-reef fish species vary considerably in the size of their geographic distributions. Some species, such as most pseudochromids (Winterbottom, 1986; Gill, 1990, unpubl. data) and many blenniids (e.g., *Ecsenius* species; Springer, 1988), have restricted distributions, while other species range widely across the region.

At present, the majority of Indo-Pacific fish species are regarded as widespread (i.e., with distributions spanning the Indo-West Pacific or greater); recent checklists give values for widespread species at between about 45 and 85% of total shorefishes, depending on locality (e.g., Russell, 1983; Gill and Reader, 1992; Winterbottom and Anderson, 1997). It seems, then, that species with narrow distributions are exceptional. However, a review of systematic literature on Indo-Pacific coral-reef fishes indicates that many apparent widespread distributions are artificial and reflect inconsistent application of criteria

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for species diagnosis. The purposes of this paper are to draw attention to these inconsistencies, and to make suggestions for future taxonomic practice. Attention is drawn, in particular, to problems associated with the use of intraspecific groupings for geographic entities within so-called widespread species.

### Criteria for intraspecific versus specific groupings

Many widespread species show geographic variation that has been well documented, but this variation has been attributed to an intraspecific level, and either recognised formally as subspecies or simply as geographic varieties. So why have workers used intraspecific categories for geographic entities rather than assigning them to separate species? Several attributes are common to most justifications for recognising variation at an intraspecific rather than specific rank. These are: 1) complete or nearly complete allopatry; 2) low degree of difference; 3) nature of the characters distinguishing forms; and 4) presence of presumed hybrids. These criteria apparently have their roots in the following assumptions: that complete or partial allopatry of populations normally precedes speciation; that geographic varieties and subspecies are allopatric populations in various stages of the process of speciation; and that the stage that they are at in this process can be inferred from the level of morphological divergence between populations and from the populations' ability to interbreed with each other.

#### *Allopatry*

Allopatry does not serve well as a criterion for distinguishing intraspecific forms from full species. Even a superficial perusal of systematic literature will reveal many pairs or groups of related species with allopatric distributions. For example, the sister species *Pseudochromis sankeyi* and *P. fridmani* are allopatrically distributed in the Red Sea (Lubbock, 1975; Gill, 1990, unpubl. data). The integrity of these two species has never been questioned. Allopatry alone is not, therefore, evidence for intraspecific rank.

#### *Low degree of difference*

How about the second criterion, low degree of difference? The first thing that comes to mind is, « How much difference is a low degree? » Some authors have suggested various measures, such as the ability to correctly classify some percentage of individuals in a sample (e.g., the « 75 percent rule », see Mayr, 1969: 190). This approach does not appear to be particularly meaningful. Firstly, and most importantly, the critical percentage values are entirely arbitrary, and it does not seem reasonable to argue that the ability to correctly classify some percentage (e.g., 75%) of individuals indicates subspecies, but some higher value (e.g., 80%) indicates full species. Secondly, it may not be meaningful to equate ability to correctly classify some percentage of a population with degree of divergence. Consider, for example, members of the *Pseudochromis tapeinosoma* group, a clade of species that are diagnosed primarily by coloration characters (Gill and Allen, 1996). Males of the five included species have highly divergent colour patterns and are easily identified, whereas females and juveniles of all species are generally brown to grey and difficult to separate from each other, particularly once preserved. Juveniles and females far outnumber males in museum samples, possibly as a result of protogynous hermaphroditism. Therefore, the percentage of correctly classified specimens using the primary character, male coloration, is low. There seems little doubt that the species are distinct. They are mostly allopatric or nearly allopatric, but two species, *P. cyanotaenia* and *P. tapeinosoma*, are broadly sympatric, although perhaps not syntopic, with no evidence of intergrades or hybrids.

It seems that ichthyologists generally have appreciated these difficulties in measuring degree of divergence with arbitrary measures. In any case, my literature review indicates that most marine Indo-Pacific fish subspecies and geographic varieties have been diagnosed by non-overlapping or at most weakly overlapping characters. Low degree of divergence has been replaced by the related third criterion, nature of the characters distinguishing the forms.

#### *Nature of characters*

The view that certain characters are informative about species and others are not is widely believed by ichthyologists. For example, throughout my training and career I have been told to not recognise species solely on the basis of coloration. More recently, I have heard concern about the sole use of morphological characters, that there is a need to check morphology with molecular characters. Such preconceived claims about the special nature or variability of certain characters over others are unfounded. There is no way of accurately predicting the hierarchical position or variability of any character type. For example, many phylogenetic studies of fish taxa have revealed large clades that are diagnosed by coloration characters. A huge clade consisting of all African cichlids except *Heterochromis* and *Tylochromis* is diagnosed by two synapomorphies, one of which is a coloration character: well-developed, strongly pigmented opercular spot (Stiassny, 1991). Conversely, externally similar species may differ in osteological characters, such as the three species in the pseudochromid genus *Ogilbyina*, which differ widely in the pattern of insertion of dorsal-fin pterygiophores between anterior neural spines (Gill, 1990). This contrasts with the prevailing notion that osteological features are conservative, and vary only among distant relatives.

The members of an undescribed pseudochromid genus provide an interesting example. The genus includes six more-or-less allopatrically distributed species, which are mainly distinguished only by coloration. Five of the species are described (and currently placed in the genus *Pseudochromis*): "*P.*" *aurifrons* (New Guinea), "*P.*" *diadema* (South China Sea, western and southern Sulu Sea), "*P.*" *ephippiatus* (Papua New Guinea and northern Sulawesi), "*P.*" *paccagnellae* (Timor Sea, eastern Indonesia, New Guinea and the Solomon Islands) and "*P.*" *porphyreus* (northeastern Indonesia, eastern Philippines, Taiwan, southern Japan and Melanesia to the Marshall Islands and Samoa). The integrity of these species, some of which are well-known aquarium fishes, has never been questioned. The final species in the genus is undescribed and known from the Great Barrier Reef and New Caledonia. It is unusual in that it differs from congeners in non-coloration characters, particularly in having relatively high numbers of scales in lateral series (Table I). How-

Table I. - Frequency distribution of bilateral counts of scales in lateral series for species within an undescribed pseudochromid genus.

	31	32	33	34	35	36	37	38	39	40	41	42	n	mean	S.D.
sp.	-	-	-	-	-	1	4	11	29	24	12	9	90	39.6	1.3
<i>aurifrons</i>	-	-	-	-	-	2	-	-	-	-	-	-	2	36.0	0.0
<i>diadema</i>	1	2	2	8	9	9	4	-	-	-	-	-	35	34.9	1.5
<i>ephippiatus</i>	-	-	-	-	1	1	2	-	-	-	-	-	4	36.3	1.5
<i>paccagnellae</i>	-	-	6	11	29	47	27	13	3	-	-	-	136	35.9	1.3
<i>porphyreus</i>	-	-	1	8	14	23	25	11	1	1	-	-	84	36.3	1.3

ever, I have encountered concern (and anticipate to encounter further concern) from ichthyologists about the validity of the species. This concern stems from the similarity in live coloration between the new species and "*P.*" *paccagnellae*; both species are bright pink anteriorly and bright yellow posteriorly. [Compare figures of the new species (misidentified as *paccagnellae*) in Power (1969), Coleman (1981) and Gill (1993: 50), with figures of *paccagnellae* in Axelrod (1973), Lubbock and Randall (1978), Kuitert (1992), Gill (1993: 53), and Kuitert and Debelius (1994).] This reaction is ironic, as, given the usual response to coloration characters, one would anticipate concern about the validity of the species that are distinguished only by coloration.

The similarities in live coloration are irrelevant to the question of whether the putative new species and "*P.*" *paccagnellae* are conspecific. Similarity in one character (coloration) does not overrule difference observed in another character (scales in lateral series). The only data that have any bearing on the apparent difference in the scale-count character are those that would lead to a different understanding of that character (e.g., from additional samples indicating a lack of significant difference in the scale count frequencies). Moreover, there is some evidence that the pink and yellow bicoloration is primitive within pseudochromids, and therefore uninformative of a close relationship between the two species. My analyses indicate that the undescribed genus is the sister group of a clade consisting of all other pseudochromids (Gill, 1990, unpubl. data). I have been unable to unequivocally relate pseudochromids to any other families, but some characters suggest a close relationship with the West Atlantic family Grammatidae (Gill and Mooi, 1993). The Grammatidae includes two genera, *Lipogramma* and *Gramma*, and the basalmost species in each genus (*L. klayi*, *G. loreto* and *G. sp.*) have pink and yellow bicoloration (Gill and Mooi, unpubl. data). This coloration is remarkably similar to that of the new species and "*P.*" *paccagnellae*, suggesting that it is primitive within the Pseudochromidae.

### Hybrids

This brings us to the final criterion, the presence of presumed hybrids. Various workers have relied on this criterion for making decisions about intraspecific versus specific classifications. For example, Randall and Heemstra (1991), synonymised the nominal allopatric species *Cephalopholis urodeta* (Pacific Ocean) and *C. nigripinnis* (Indian Ocean) partly because of the discovery of four probable hybrids at Christmas Island in the eastern Indian Ocean. (They also cite the lack of non-coloration characters as justification for not recognising full species.) The hybridization criterion has its roots in the biological species concept, which contends that the ability of individuals to reproduce with each other is the primary test of a species. Space constraints do not allow adequate discussion of species concepts, and the issues I want to raise in this paper are concerned with practices that are largely independent of species concepts. Nevertheless, I believe the biological species concept has been convincingly dismissed by others, in particular by Rosen (1979). Rosen argued that the ability for individuals to reproduce with each other is a primitive attribute (symplesiomorphy) and not indicative of close relationship let alone a test of species. Rosen provided examples of natural hybridization between various unrelated poeciliid fish species.

There are relatively few reported cases of natural hybridization in marine fishes (Slattenenko, 1957; Schwartz, 1972, 1981), and those that have been reported do not necessarily involve closely related parent species. For example, 12 probable hybrid crossings have been reported for the family Chaetodontidae: *Chaetodon aureofasciatus* x *C. rainfordi*, *C. auriga* x *C. ephippium*, *C. auriga* x *C. fasciatus*, *C. auriga* x *C. lunula*, *C.*



*ephippium* x *C. semeion*, *C. ephippium* x *C. xanthocephalus*, *C. kleini* x *C. unimaculatus*, *C. meyeri* x *C. ornatissimus*, *C. milliariis* x *C. multicinctus*, *C. milliariis* x *C. tinkeri*, *C. ocellatus* x *C. striatus* and *C. pelewensis* x *C. punctatofasciatus* (Burgess, 1974, 1978; Randall *et al.*, 1977; Allen, 1981; Randall and Fridman, 1981; Clavijo, 1985). Of these pairings, only five (*C. aureofasciatus* x *C. rainfordi*, *C. ephippium* x *C. xanthocephalus*, *C. meyeri* x *C. ornatissimus*, *C. ocellatus* x *C. striatus* and *C. pelewensis* x *C. punctatofasciatus*) probably involve sister parent species (determined from the classification in Blum, 1989: Table I). Similarly, in cases from other marine fish families, natural putative hybrids have involved parent species from different genera [e.g., *Cephalopholis fulva* x *Paranthias furcifer* (Serranidae), Smith, 1966, Thompson and Munro, 1978; *Liopsetta putmani* x *Pseudopleuronectes americanus* (Pleuronectidae), Hoornbeek and Klein-Macphée, 1987; *Lutjanus synagris* x *Ocyurus chrysurus* (Lutjanidae), Loftus, 1992; *Lutjanus griseus* x *Ocyurus chrysurus* (Lutjanidae), Domeier and Clarke, 1992; *Paracentropyge multifasciatus* x *Sumieryakko venustus* (Pomacanthidae), Krupp and Debelius, 1990 (however, see Pyle and Randall, 1992, for justification for the classification of both parent species in *Centropyge*)]. Clearly, hybridization is not a valid criterion for distinguishing between specific and intraspecific forms.

In short, none of the criteria currently used for distinguishing intraspecific forms from full species is justified.

#### Additional reasons for rejecting intraspecific groupings

There are several additional reasons for objecting to the use of intraspecific categories. These pertain to 1) the subsequent usage of such categories, 2) the phylogenetic relationships that are implied by the groupings, and 3) the influence of the groupings on nomenclatural stability.

#### Subsequent usage of intraspecific groupings

One of the primary reasons for objecting to the use of intraspecific categories is that they are rarely cited in literature on Indo-Pacific fishes. For example, Burgess (1978) recognised three butterflyfish species with allopatrically distributed subspecies: *Chaetodon trifasciatus*, with subspecies *trifasciatus* (Indian Ocean) and *lunulatus* (Pacific Ocean; recently recognised as a separate species by Kuitert, 1994); *C. unimaculatus*, with subspecies *unimaculatus* (Pacific Ocean) and *interruptus* (Indian Ocean); and *C. vagabundus*, with subspecies *vagabundus* (Indo-Pacific except southern Arabia) and *pictus* (southern Arabia). Numbers of references to these species in a selected survey of literature (mainly checklists and field guides; full reference list available on request) are given in table II. Subspecific categories are indicated in only 10 out of 82 total references to the species (12%). Therefore, new information on the morphology, biology and distribution

Table II. - Numbers of references to *Chaetodon* subspecies proposed by Burgess (1978) based on a review of 30 publications (mainly checklists and field guides).

	<i>trifasciatus</i>	<i>unimaculatus</i>	<i>vagabundus</i>	Totals
Total number of references to species	28	27	27	82
References that differentiate subspecies (% of total)	5 (18)	5 (19)	0 (0)	10 (12)

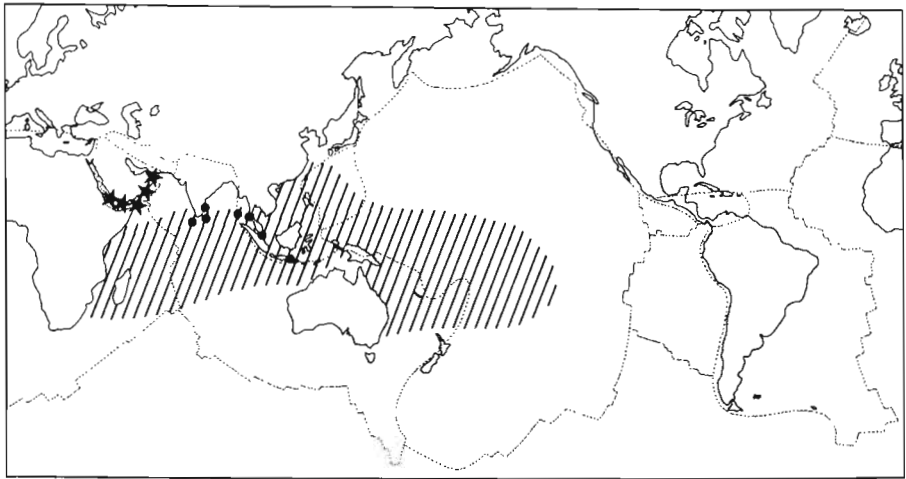


Fig. 1. - Distribution of the butterflyfishes *Chaetodon decussatus* (dots), *C. vagabundus pictus* (stars) and *C. v. vagabundus* (hatched area).

of these forms has not accumulated. Surprisingly, references that do not distinguish sub-specific forms include Allen's (1981) popular review of chaetodontids and pomacanthids and Blum's (1989) important biogeographical analysis of the Chaetodontidae.

The situation is even worse for informal intraspecific categories such as "geographic varieties". For example, Burgess (1976) reported on two widespread species with different colour forms in the Pacific and the Indian Ocean, one of which was the triggerfish *Balistapus undulatus*. Pacific and eastern Indian Ocean specimens of *B. undulatus* are readily distinguished from western and central Indian Ocean specimens by coloration of the caudal fin (yellow to orange, dark on upper and lower margins, with undulating blue to grey stripes extending from base of fin, versus without undulating stripes) and caudal peduncle (large black blotch surrounding peduncular spines, versus black stripe along each row of spines). Despite the fact that the two forms of *Balistapus* are well diagnosed, I have been unable to locate any subsequent references that differentiate between them.

#### *Implications of intraspecific hierarchy*

Another difficulty with the use of intraspecific groupings is that they imply phylogenetic (and associated biogeographic) relationships that may be unsupported by available evidence (i.e., synapomorphies). Consider, for example, the above-mentioned subspecies of *Chaetodon vagabundus*, *pictus* from southern Arabia and *vagabundus* from elsewhere in the Indo-Pacific (Fig. 1). Burgess (1978) hypothesised a close relationship between *C. vagabundus* and the central-eastern Indian Ocean species *C. decussatus*, and Blum (1988) included the two species and *C. auriga* in the subgenus *Rabdophorus* as sole members of his *C. auriga* group. Burgess distinguished the two subspecies of *C. vagabundus* primarily on the basis of coloration characters, most notably the presence of mostly black dorsal-fin spines in *pictus* (compare figures of *pictus* provided by Burgess, 1978: 416, and Randall, 1995: 252, with those of *vagabundus* provided by Burgess, 1978: 412-413, and Steene, 1978: 52). However, *C. decussatus* also has mostly black dorsal-fin spines (see Burgess, 1978: 416-417, and Allen, 1981: 178-179); *C. auriga*, the remaining species in the *C. auriga* group, lacks mostly black spines, as do most of the 27 species

recognised in the subgenus *Rabdophorus* (exceptions include *C. auripes*, *C. collare*, *C. dialeucos*, *C. fasciatus*, *C. flavirostris*, *C. lunula*, *C. mesoleucos*, *C. nigropunctatus* and *C. wiebeli*). Therefore, mostly black spines may be interpreted as a synapomorphy between *pictus* and *decussatus*, a relationship predicted by biogeographic patterns shown by some other taxa in the region (such as *Pseudochromis persicus* and its relatives; Gill, 1990, unpubl. data).

#### *Nomenclatural stability*

I believe that the use of subspecific categories will generally lead to instability of nomenclature. This is because different studies may support different relationships among taxa. Consider, for example, the above case involving the nominal *Chaetodon* species, *decussatus*, *pictus* and *vagabundus*. It is conceivable that some authors may elect to include *pictus* as a subspecies of *decussatus*, while others may retain it with *vagabundus*. Given current practice, the species would then be reported as either *C. decussatus*, *C. d. pictus*, *C. vagabundus* or *C. v. pictus* ! This instability would be avoided if *pictus* (and other intraspecific forms) were elevated to full species.

## CONCLUSIONS

As a concluding remark, I would like to comment on the prevailing notion among ichthyologists that, for the most part, Indo-Pacific fish species and their distributions are fairly well known. I believe that this notion is false. In particular there is a need for careful reevaluation of the numerous so-called widespread species. I anticipate that this "careful reevaluation" will dominate future systematic research in the region. The task is far from simple, and may exceed previous systematic progress in required effort and degree of difficulty. Sadly, it comes at a time when, despite political interests in biodiversity, funding support for systematics, particularly morphology based systematics, is reducing on a global scale. Nevertheless, I believe that recognition of well-diagnosed geographic forms as full species rather than subspecies or geographic varieties will be an important first step in the task of dealing with widespread species. Clearly, the current practice of arbitrarily allocating geographic forms to intraspecific ranks is both unjustified and misleading.

**Acknowledgements.** - In preparing this paper I have benefitted from discussions with others, including R. Bruce, K. Carpenter, M. de Carvalho, J. Kemp, R.D. Mooi, T. Munroe, G. Nelson, J.E. Randall, D.J. Siebert, V.G. Springer and J.T. Williams; the views expressed, however, remain my own. Drafts of the manuscript were improved from comments by N. Merrett, R.D. Mooi, D.J. Siebert and R. Winterbottom.

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**PRELIMINARY STUDY OF THE MORPHOLOGY OF  
THE HEAD IN POMACENTRIDAE:  
ADDUCTOR MANDIBULAE ORGANIZATION IN  
*DASYLLUS ARUANUS* (TELEOSTEI: PERCIFORMES)**

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**ABSTRACT.** - The family Pomacentridae contains a great number of species, living mostly on coral reefs. This diversity could be explained by the variation of ecological characteristics. There are few differences in external morphology that could explain this richness among damselfishes. One way to approach this problem is to look for differences in the organization of internal structures. We chose to start the study with the examination of the adductor mandibulae of *Dascyllus aruanus*, species widely distributed in tropical and sub-tropical waters of the Indo-Pacific. This muscle exhibits a remarkable complexity, more so than the variation previously described for the perciforms. With the different sections (A1, A2, A3 and A $\omega$ ), *D. aruanus* shows many original subdivisions, mainly in A2 and A3 and their insertions.

**RÉSUMÉ.** - Étude préliminaire de la morphologie de la tête des Pomacentridae: l'organisation de l'adductor mandibulae chez *Dascyllus aruanus* (Teleostei: Perciformes).

La famille des Pomacentridae comprend un grand nombre d'espèces qui vivent pour la plupart dans les récifs coralliens. Cette biodiversité pourrait être expliquée par la grande variabilité des niches écologiques. Il existe très peu de différences dans la morphologie externe qui pourraient être le reflet de la richesse spécifique. Le but de cette étude est d'examiner les différences dans l'organisation des structures morphologiques liées à la prise de nourriture. Nous avons choisi de commencer ce travail par l'observation du muscle adducteur de la mandibule chez *Dascyllus aruanus*, une espèce largement répandue dans les eaux tropicales et sub-tropicales de la zone indo-pacifique. Ce muscle montre une complexité remarquable, plus importante que celle qui a été décrite jusqu'à présent chez les Perciformes. On retrouve d'une part les différentes sections habituellement décrites, à savoir A1, A2, A3 et A $\omega$ , et d'autre part de nombreuses originalités, notamment dans la division en sous-faisceaux de A2 et A3.

**Key-words.** - Pomacentridae, *Dascyllus aruanus*, Morphology, Adductor mandibulae.

Among the higher teleost fishes, especially perciforms, the division of the adductor mandibulae muscle is variable, going from two divisions to the individualization of four main divisions A1, A2, A3 and A $\omega$ , themselves sometimes being divided (Gosline, 1971; Winterbottom, 1974). This partitioning is observed in the Cichlidae (Chardon and Vandewalle, 1971; Vandewalle, 1972; Liem and Osse, 1975; Liem, 1978, 1979; Stiassny, 1981), Embiotocidae (Stiassny, 1981) and Labridae (Van Hasselt, 1978, 1979a, 1979b;

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Stiassny, 1981; Westneat, 1990) and also in the Pomacentridae following Ciardelli (1967) and Stiassny (1981).

The variability of the origins and insertions of different divisions is frequently used to support hypotheses about phyletic relations (Rosen and Patterson, 1969; Gould and Eldredge, 1977; Stiassny, 1981; Gosline, 1986). It is also used in ecomorphological studies that tend to focus on the relations between structure and function (Osse, 1969; Liem and Osse, 1975; Liem, 1978, 1979; Gosline, 1981, 1987; Motta, 1982; Dutta, 1987; Turingan, 1994). A precise description of this musculature can be useful for the comprehension of diet diversity (Schaeffer and Rosen, 1961; Ciardelli, 1967; Elshoud-Oldenhave, 1979; Liem, 1978, 1979).

The Pomacentridae contains at least 320 species found mostly in tropical and sub-tropical waters (Allen, 1975, 1991). All these species show a similar external morphology that contrasts with the diversity of habits and diet (Randall, 1967; Emery, 1973; Allen, 1975, 1991; Coates, 1980; Montgomery, 1980). Thus, it seems interesting to study the skeleton and muscle morphology of the feeding apparatus. It is in this way that the present work on the adductor mandibulae is realized. This completes the first observations of the head osteology of *Dascyllus aruanus* (Gluckmann and Vandewalle, in press).

We chose *D. aruanus* (Linnaeus, 1758) (Pomacentridae, Chrominae) because it is omnivorous, mainly feeding on small pelagic prey (Randall, 1967; Allen, 1991). This species is widely distributed in tropical and sub-tropical waters and is already studied from an ecological (Sweetman, 1983; Sikkil, 1995) and genetic perspective (Planes *et al.*, 1993).

The nomenclature of the adductor mandibulae is based upon Liem (1970), Winterbottom (1974) and Stiassny (1981).

## MATERIAL AND METHODS

The specimens of *D. aruanus* (21 to 42 mm long) were collected on Laing's Island (Bismarck Sea, Papua New-Guinea) and in the lagoon of the north coast of Moorea (Society Islands, French Polynesia).

Anatomical descriptions were made from observations of specimens fixed in 5% formalin, and stored in 75% ethyl alcohol and dissected under a binocular WILD M5 microscope. Drawings were made with the aid of a camera lucida.

## RESULTS

The adductor mandibulae is divided in four main divisions and nine subdivisions.

A1 originates from the vertical limb of the preopercular and the hyomandibular. Fibers, originally parallel, converge to a tendon (tA1) inserting on the medial face of the maxilla, just under its premaxillary process (Fig. 1).

A2 is composed of two triangular shaped subdivisions. A2 $\alpha$ , the most lateral part, originates from the crescentic zone of the horizontal limb of the preopercular, the quadrate and the symplectic (Fig. 1). The deeper A2 $\beta$  arises from the quadrate (Fig. 2A). The tendons that extend from those two parts merge and pass laterally to the angulo-articular which fits to the lateral face of the coronoid process of the dentary (Fig. 1).



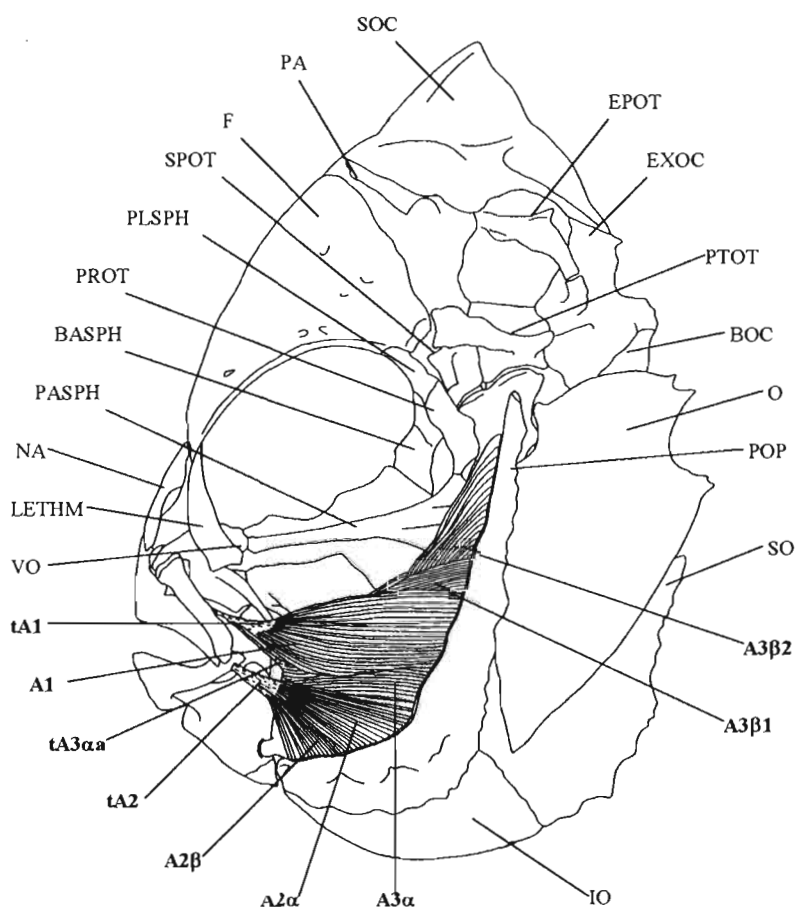


Fig. 1. - Left lateral view of the neurocranium, suspensorium and adductor mandibulae muscles of *Dascyllus aruanus*. A1-A3β2: portions of the adductor mandibulae, BASPH: basisphenoid, BOC: basioccipital, EPOT: epiotic, EXOC: exoccipital, F: frontal, IO: interopercular, LETHM: lateral ethmoid, NA: nasal, O: opercular, PA: parietal, PASPH: parasphenoid, PLSPH: pleurosphenoid, POP: preopercular, PROT: prootic, PTOT: pterotic, SO: subopercular, SOC: supraoccipital, SPOT: sphenotic, tA1-tA3α: tendon of the adductor mandibulae-A1-A3α, VO: vomer.

A3 is composed of two divisions. A3β is subdivided. A3α is thin and appears between A1 and A2 (Figs 1, 2A,B). It extends from the preopercular and the hyomandibular to the anterior aponeurosis. Its fibers are parallel from the origin to this aponeurosis. On that spot, dorsal fibers extend in a thin tendon (tA3αa) that merges with tA1 (Fig. 1), while ventral fibers extend downwards into a tendon (tA3αb) that first merges with tA3β, then separates from it and passes along the medial face of the angulo-articular (Figs 2B, 4). The most inner fibers mix with those of the subdivision A3β1. The tA3αb is extended by the Aω2 division that arises from the internal face of the angulo-articular and the dentary (Fig. 4). A3α and Aω2 form a two headed muscle intervened by a tendon.

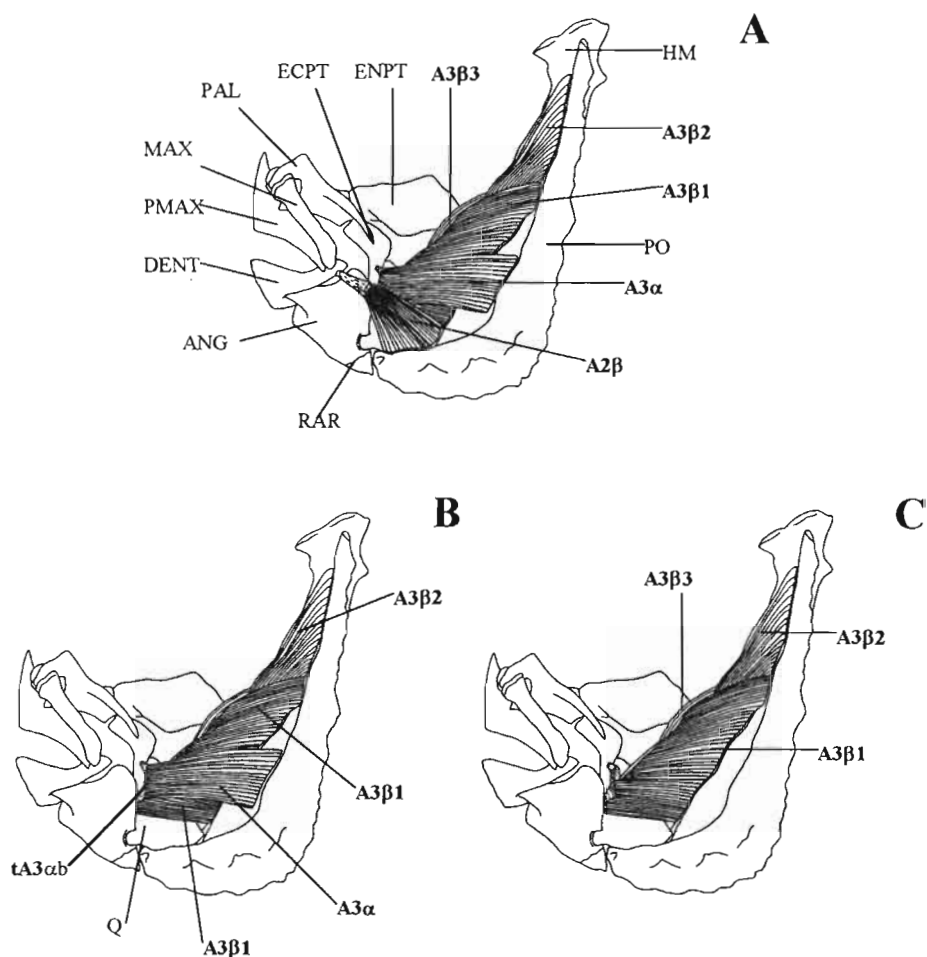


Fig. 2. - Left lateral view of the suspensorium and adductor mandibulae muscles of *Dascyllus aruanus*. A. After removal of A1 and A2 $\alpha$ . B. After removal of A1, A2 $\alpha$  and A2 $\beta$ . C. After removal of A1, A2 $\alpha$ , A2 $\beta$  and A3 $\alpha$ . A2 $\beta$ -A3 $\beta$ 3: portions of the adductor mandibulae, ANG: angulo-articular, DENT: dentary, ECPT: ectopterygoid, ENPT: entopterygoid, HM: hyomandibular, MAX: maxillar, PAL: palatine, PMAX: premaxillar, POP: preopercular, Q: quadrate, RAR: retroarticular, tA3 $\alpha$ b: tendon of the adductor mandibulae-A3 $\alpha$ .

The subdivisions of A3 $\beta$  are recognizable by their fiber orientation. They all insert on the developed tA3 $\beta$  (Figs 2C, 3). The fibers of the most lateral subdivisions A3 $\beta$ 1 arise from the symplectic, the quadrate, the metapterygoid, the hyomandibular, the preopercular and insert on the external side of tA3 $\beta$ . Some of the fibers situated under A3 $\alpha$  mix with this one. The subdivision A3 $\beta$ 2 originates from the dorsal part of the hyomandibular and the preopercular. Those fibers converge to attach themselves to the extremity of tA3 $\beta$ . The most median section, A3 $\beta$ 3, arises from the metapterygoid and the ventral part of the hyomandibular flange. It inserts on the inner side of tA3 $\beta$ , near the insertion of dorsal division A3 $\beta$ 1. The tA3 $\beta$  inserts on the medial face of the lower jaw, on the coroneomeckelien (Fig. 4).

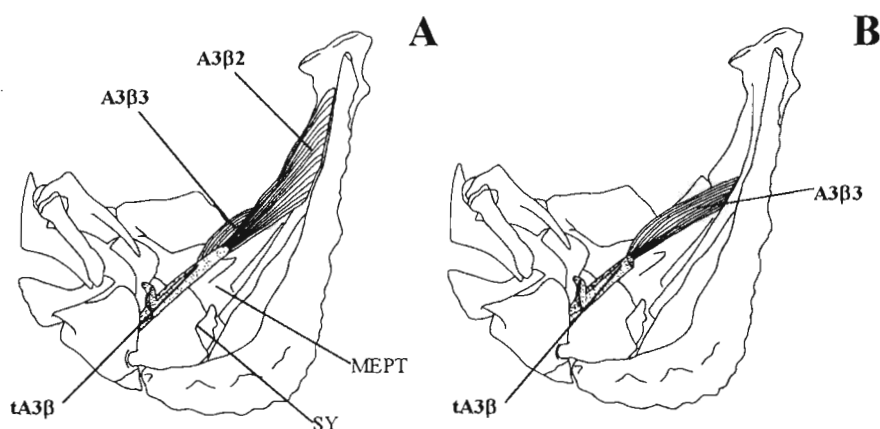


Fig. 3. - Left lateral view of the suspensorium and adductor mandibulae muscles of *Dascyllus aruanus*. A. After removal of A1, A2 $\alpha$ , A2 $\beta$ , A3 $\alpha$  and A3 $\beta$ 1. B. After removal of A1, A2 $\alpha$  and A2 $\beta$ . C. After removal of A1, A2 $\alpha$ , A2 $\beta$ , A3 $\alpha$ , A3 $\beta$ 1 and A3 $\beta$ 2. A3 $\beta$ 2-A3 $\beta$ 3: portions of the adductor mandibulae, MEPT: metapterygoid, SY: symplectic, tA3 $\beta$ : tendon of the adductor mandibulae-A3 $\beta$ .

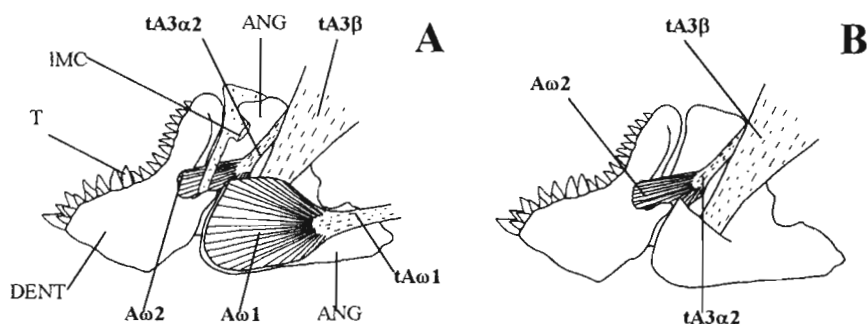


Fig. 4. - A. Medial view of the lower jaw of *Dascyllus aruanus*. B. After removal of A $\omega$ 1. A $\omega$ 1- A $\omega$ 2: portions of the adductor mandibulae, ANG: angulo-articular, DENT: dentary, LMC: mandibulo-ceratothyal ligament, T: teeth, tA3 $\alpha$ 2-tA3 $\beta$ -tA $\omega$ 1: tendon of the adductor mandibulae-A3 $\alpha$ 2-A3 $\beta$ -A $\omega$ 1.

A $\omega$ 1 originates on the medial face of the angulo-articular. It partially covers A $\omega$ 2 and tA3 $\beta$ . Its fibers converge posteriorly in a tendon tA $\omega$ 1, which inserts on the medial face of the quadrate and the preopercular (Fig. 4A).

## DISCUSSION

The variability of the different divisions of the adductor mandibulae in perciforms is a character often used in a taxonomy to establish the relationship between the different groups (Rosen and Patterson, 1969; Gould and Elderedge, 1977; Stiassny, 1981; Gosline, 1986). The only constant fact is the partitioning of the adductor into two divisions, the most developed one constituting the cheek muscles (A1, A2, A3) and a smaller part, situated on the medial face of the lower jaw (A $\omega$ ) (Winterbottom, 1974; Gosline, 1986). Indi-

vidual variation among species is associated with the evolution of jaws protrusion in acanthopterygian fishes (Osse, 1969). The multiplicity of these divisions could be bound to the various possibilities of modulation in the mouth opening in higher perciforms (Liem, 1993).

The lower perciforms, such as the nandid *Polycentrus schomburgkii*, show only one division AM inserted on the maxilla and the medial face of the lower jaw, and the division A $\omega$  (Liem, 1970).

In the higher perciforms, derived taxa have progressively more divisions. In some Serranidae (Alexander, 1967; Stiassny, 1981; Benmouna *et al.*, 1984a; Vandewalle *et al.*, 1986), Embiotocidae (Stiassny, 1981), Mullidae (Vandewalle *et al.*, 1986), two divisions are clearly separated anteriorly. The dorsal division inserts on the maxilla and the ventral division on the lower jaw. The dorsal part is named A1 and the ventral one, A2,3. In other species, as the nandid *Nandus nebulosus* (following Liem, 1970) and some chaetodontids (Motta, 1982), there is a lateral division inserting on the maxilla and the lower jaw, and a medial division inserting only on the lower jaw. The lateral part is named A1A2, and the medial one, A3.

In the Percidae (Osse, 1969; Elshoud-Oldenhave, 1979) and Centrarchidae (Vandewalle *et al.*, 1986), the distinction between A2 and A3 becomes clearer. In Cichlidae (Chardon and Vandewalle, 1971; Liem and Osse, 1975; Liem, 1978, 1979; Stiassny, 1981; Vandewalle, 1972), Labridae (Stiassny, 1981; Van Hasselt, 1978, 1979a, 1979b; Westneat, 1990) and the Sparidae (Vandewalle *et al.*, 1995; Hernandez and Motta, 1997), the divisions A1, A2 and A3 are clearly separated.

The A1 section always inserts on the maxilla, by one or more tendon (t). In many species, tA1 attaches on the medial face of this bone. We found this situation in the Nandidae (Liem, 1970), the Chaetodontidae (Motta, 1982), the Embiotocidae (Stiassny, 1981), the Centrarchidae (Vandewalle *et al.*, 1986), the Pomacentridae (Ciardelli, 1967; Stiassny, 1981) and the Cichlidae (Chardon and Vandewalle, 1971; Vandewalle, 1972; Liem and Osse, 1975; Liem, 1978, 1979; Stiassny, 1981). Westneat (1990) described the same type of insertion in the Labridae. Otherwise, Stiassny (1981) described an insertion on the edge of the premaxillary process of the maxilla, which she interprets as a synapomorphy of the family. In the Percidae (Osse, 1969; Elshoud-Oldenhave, 1979), A1 inserts on the lateral face of the maxilla, whereas, in the Serranidae (Alexander, 1967; Stiassny, 1981; Benmouna *et al.*, 1984a) and the Sparidae *Diplodus sargus* (Vandewalle *et al.*, 1986; Vandewalle *et al.*, 1995), it inserts on the lateral and the medial face of this bone. Usually, A1 does not only insert on the maxilla. In the Cichlidae (Chardon and Vandewalle, 1971; Vandewalle, 1972; Liem and Osse, 1975; Liem, 1978, 1979; Stiassny, 1981), a tendon attaches to the medial face of the lower jaw. In some mullids (Vandewalle *et al.*, 1986), percids (Osse, 1969; Elshoud-Oldenhave, 1979), serranids (Alexander, 1967; Stiassny, 1981; Benmouna *et al.*, 1984a), centrarchids (Vandewalle *et al.*, 1986), labrids (Van Hasselt, 1978, 1979a, 1979b; Stiassny, 1981; Westneat, 1990) and the sparid *D. sargus* (Vandewalle *et al.*, 1986; Vandewalle *et al.*, 1995), a tendon arises from A1 to tA3. In the sparid *Archosargus probatocephalus*, a tendon arises from A1 to tA2 (Hernandez and Motta, 1997). Following Stiassny (1981), fibers of ventral portion of A1 insert aponeurotically on the lateral face of the dentary in labrids.

In many species, A2 inserts on the medial face of the ascending process of the angulo-articular. This situation is not common. In the Sparidae *D. sargus* (Vandewalle *et al.*, 1995), A2 inserts on the medial face of the angulo-articular and the dentary. In the Labridae (Van Hasselt, 1978, 1979a, 1979b; Stiassny, 1981; Westneat, 1990) and the

Pomacentridae (Ciardelli, 1967; Stiassny, 1981), this division inserts on the lateral face of the coronoid process of the dentary. Stiassny (1981) determined it to be A1 $\beta$ . The medial division then being named A2,3. It seems to be more usual to name this division A2. The deeper one is named A3, following Winterbottom (1974) and Van Hasselt (1978, 1979a, 1979b). In *D. sargus*, A2 has a tendon directly attached on the maxillary (Vandewalle *et al.*, 1995). This situation is not described in the sparid *A. probatocephalus* (Hernandez and Motta, 1997).

In many perciforms, A3 inserts at least on the coronomeckelien, except in the Nandidae. In these fishes, AM in *P. scomburgkii* and A3 in *N. nebulosus* converge into a tendon extending past A $\omega$  (Liem, 1970). In many perciforms, A3 inserts on the coronomeckelien and is connected with A $\omega$  (Serranidae (Alexander, 1967; Stiassny, 1981; Benmouna *et al.*, 1984a), Mullidae (Vandewalle *et al.*, 1986), Sparidae (Vandewalle *et al.*, 1986; Vandewalle *et al.*, 1995), Embiotocidae (Stiassny, 1981), Centrarchidae (Vandewalle *et al.*, 1986), Labridae (Van Hasselt, 1978, 1979a, 1979b; Stiassny, 1981; Westneat, 1990) and Pomacentridae (Ciardelli, 1967; Stiassny, 1981)). In the Chaetodontidae (Motta, 1982), the Cichlidae (Chardon and Vandewalle, 1971; Vandewalle, 1972; Liem and Osse, 1975; Liem, 1978, 1979; Stiassny, 1981) and the Percidae (Osse, 1969; Elshoud-oldenhove, 1979), there is no connection between A3 and A $\omega$ . In *D. sargus* (Vandewalle *et al.*, 1995), a tendon arises from A3 and inserts on the maxilla.

A $\omega$  is often formed by a single division, which covers a large part of the dentary and angulo-articular medial faces. However, it can be less extensive: in the Nandidae *N. nebulosus*, it only covers the dentary (Liem, 1970), and in the Sparidae (Vandewalle *et al.*, 1986) and the Pomacentridae (Ciardelli, 1967; Stiassny, 1981), it only inserts on the angulo-articular. Moreover, in *D. sargus*, it is divided into two subdivisions, A $\omega$  and A $\omega'$  (called A3' in Vandewalle *et al.*, 1995). A $\omega$  is related to the medial face of the quadrate and A $\omega'$  is related to tA3.

The adductor mandibulae in *D. aruanus* shows is more complex than those described until now in other perciforms. With the different divisions described (A1, A2, A3 and A $\omega$ ), *D. aruanus* shows many novelties in the divisions, mainly in A2 and A3 and in their insertions. A good careful examination of the complexity of the cranial musculature, and more particularly of the adductor mandibulae, could explain how the remarkable adaptive radiation of the Pomacentridae has been formed.

**Acknowledgements.** - We would like to thank S. Houbart and C. Michel (Aquarium Dubuisson, Liège, Belgium), J-M Ouin and G. Seghers (Laing Island Biological Station, Papua New-Guinea) and Y. Chancerelle and J. Algret (CRIOBE, Moorea, French Polynesia) for helping with obtaining specimens of the Pomacentridae. This work is supported by Grant n°2.4560.96 from the Belgian « Fond National de la Recherche Scientifique ».

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## BIOGEOGRAPHY OF THE NEARSHORE MARINE FISH FAUNA OF THE KIMBERLEY, WESTERN AUSTRALIA

by

J. Barry HUTCHINS (1)

**ABSTRACT.** - The marine fauna of the Kimberley, Western Australia, was surveyed on four occasions during the years 1991-1996. This produced a total of 469 species, 70% of which have ranges extending well outside the Australian region. Species shared with the remainder of tropical Australia accounted for 8% of the total, while 5% are species shared between Western Australia and the Northern Territory. Endemic Western Australian species accounted for 3%, and only 1% are confined to the Kimberley. Although many areas still remain to be visited, the results of these surveys indicate that coastal waters of the Kimberley can be divided into three faunal zones: South-west Kimberley, North Kimberley and North-east Kimberley. The south-western portion is inhabited by a fauna which shares many species with the Pilbara region to the south, whereas the north-eastern portion supports some species that are more characteristic of the Northern Territory than Western Australia. The northern portion contains all of the species that are endemic to the Kimberley, and its fauna is probably the most representative of the Kimberley area.

**RESUME.** - Biogéographie de la faune ichtyologique des côtes du Kimberley, Australie occidentale.

La région du Kimberley a été prospectée quatre fois entre 1991 et 1996. Parmi les 469 espèces de poissons recensées, 70% ont une répartition qui va bien au-delà de la région Australienne, 8% sont présentes dans toute la zone tropicale (dont fait partie le Kimberley), 5% sont observées aussi dans le Territoire du Nord, 3% sont endémiques pour l'Australie occidentale et 1% sont confinées à la région du Kimberley. Bien que plusieurs zones n'aient pas encore été prospectées, la région du Kimberley peut être subdivisée en trois aires faunistiques: Sud-Ouest, Nord et Nord-Est. La faune du Sud-Ouest est très proche de celle de la région de Pilbara au Sud. Les espèces de la zone du Nord-Est sont apparemment plus typiques du Territoire du Nord. Les espèces endémiques de la région se trouvent dans la portion Nord du Kimberley.

**Key-words.** - Ichthyofauna, ISW, Western Australia, Kimberley, Biogeography, Biodiversity, Inventory.

The Kimberley coast of Western Australia (Fig. 1) is one of the most isolated and under-populated areas of Australia. Morphologically, it ranges from rugged uplifted plateaux that have been heavily eroded to river deltas with extensive networks of mangal. Until recently, investigations of its nearshore fauna had been minimal because of the difficulty of reaching much of the region, and the inhospitable nature of its environment. Furthermore, 4-5 months of the year are usually unsuitable for fieldwork due to possibility of cyclonic weather conditions. In 1991, the Western Australian Museum conducted surveys in the region between Wyndham and Broome using a chartered vessel (Morgan,

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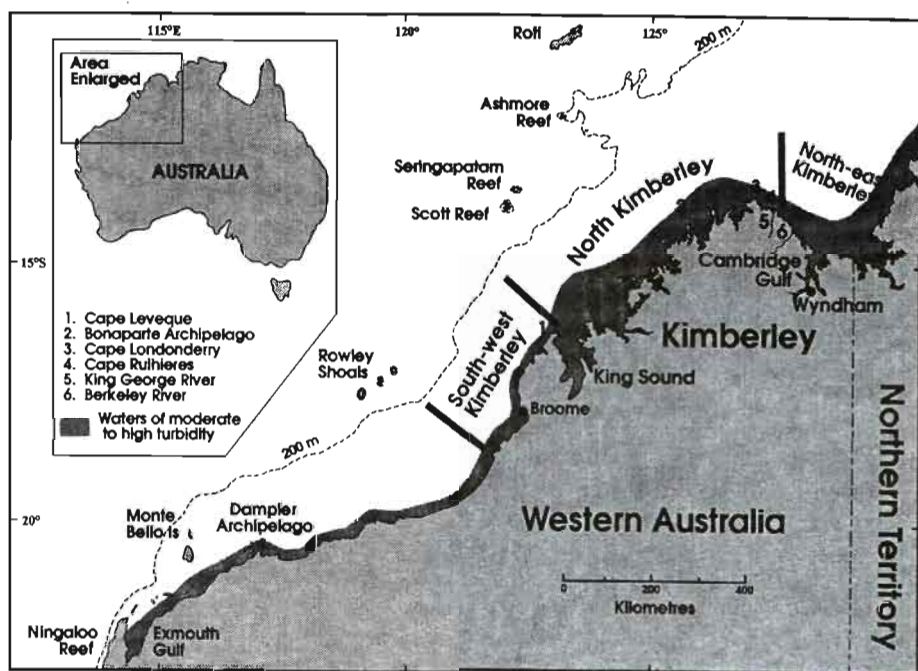


Fig. 1. - Map of tropical Western Australia showing the Kimberley and adjacent regions.

1992). This was followed by three more cruises organised jointly by the Western Australian Museum, the University of Western Australia and the Northern Territory Museum (Wells *et al.*, 1995; Walker *et al.*, 1996; Walker, 1997). The fishes collected and observed on these expeditions (Allen, 1992; Hutchins, 1995, 1996b; Morrison and Hutchins, 1997) have added greatly to our knowledge of the Kimberley's marine fauna. Even though many areas still remain unexplored, these data indicate that the nearshore Kimberley is inhabited by a fauna that generally has much in common with other nearshore areas of tropical Australia. Nevertheless some unique species assemblages help distinguish the Kimberley from its neighbours, and also aid in the division of its coastal waters into several apparent biogeographical zones (Fig. 1). The purposes of this paper are 1) to present a preliminary account of the distribution and diversity of the nearshore fishes of the Kimberley, 2) to contrast this with other areas of tropical Western Australia, and 3) briefly to compare the fishes of north-western Australia with those of north-eastern Australia.

The coastal waters of northern Australia can be broadly divided into two categories: 1) waters which are turbid for much of the year, and 2) waters which are usually of good clarity. The former are found inshore where rivers, mangroves and large tidal fluctuations contribute to the turbidity. Waters of good clarity occur offshore although these may be nearshore where river outflow is minimal. In Western Australia, the whole coastline from Exmouth Gulf northwards is bathed in waters of moderate to high turbidity (Wilson, 1994) (Fig. 1). Offshore the waters are of good clarity, although these conditions are also found inshore along the mainland coast to the south of Exmouth Gulf where river outflow is generally low. The nearshore region of the Kimberley has the highest turbidity, particularly in the vicinity of the two largest bodies of semi-enclosed waters, King Sound and

Cambridge Gulf (Brooke, 1995, 1997). Both of these areas are fed by large rivers and possess tidal fluctuations of up to 11 m. Enormous areas of tidal mud flats and mangroves are the source of much of the silt affecting these waters (Semeniuk, 1981).

The nearshore fish fauna of the Kimberley, based on collections and visual surveys made by both terrestrial and underwater observers - the latter to depths of 35 m - is briefly summarised in table I. Wide-ranging species predominate, comprising almost 70% of the 469 species recorded. The next highest category consists of species found only in tropical Australia (sometimes also ranging along the southern coastline of Papua New Guinea) with 8% of the total number. Western Australian endemics make up about 3% and are listed in table II (less than 1% are endemic to the Kimberley); this number could change as there is still considerable work to be done in some families. The families with the highest numbers of species were the Gobiidae (approx. 66 species), Labridae (34), Pomacentridae (31), Apogonidae (20), Serranidae (18), and Blenniidae (18). The majority of species recorded were associated with hard substrates (rocky/coral reefs and intertidal rockpools), although samples were taken from soft bottom habitats, and small epipelagic fishes were collected - often at night - using dipnets.

Most of the 469 species are able to tolerate waters of moderate turbidity and strong tidal currents. Many range south to Exmouth Gulf (only 75 species have not been found south of the Kimberley, see table III) and also eastwards across the Northern Territory to Queensland. However, the fauna is depauperate in comparison with the number recorded for the nearby offshore atolls of Rowley Shoals, Scott and Seringapatam Reefs, and Ashmore Reef (approx. 1,000 species [Done *et al.*, 1994]).

Table I. - Biogeographical Analysis of Kimberley Fishes.

Distribution	Number of species
Wide-ranging	320
WA, NT, Qld (sometimes to PNG)	39
WA and NT	22
WA and South-East Asia	19
WA only	15
Northern Australia and South-East Asia	10
Uncertain	44
<b>Total species</b>	<b>469</b>

Table II. - Endemic species of Western Australia recorded during this study. \*: known only from the Kimberley.

Family	Species	Family	Species
Muraenidae	<i>Gymnothorax</i> sp.*	Apogonidae	<i>Pseudanias</i> sp.
Batrachoididae	<i>Batrachomoeus dahl</i>		<i>Pterapogon mirifica</i>
Atherinidae	<i>Craterocephalus pauciradiatus</i>	Sparidae	<i>Acanthopagrus palmalis</i>
Serranidae	<i>Epinephelus bilobatus</i>	Lethrinidae	<i>Lethrinus</i> sp.
Pseudochromidae	<i>Assiculoides desmonotus</i> *	Pomacanthidae	<i>Chaetodontoplus personifer</i>
	<i>Labracinus lineatus</i>	Scaridae	<i>Scarus</i> sp.
Notograpidae	<i>Notograptus</i> sp.*	Blenniidae	<i>Cirripectes alleni</i> *
		Monacanthidae	<i>Colurodon paxmani</i>

Evidence suggests that the nearshore fishes are not distributed evenly throughout the Kimberley. While the majority of species occur on the central Kimberley coast, some species were found only in the south-west, and others only in the north-east. Analysis of these distributions allows the Kimberley coast to be divided into three zones (Fig. 1): South-west Kimberley, North Kimberley, and North-east Kimberley.

Table III. - Species not or rarely found south of the Kimberley.

Family	Species	Family	Species
Muraenidae	<i>Gymnothorax longinquus</i> <i>Gymnothorax pseudothyroideus</i> <i>Gymnothorax</i> sp. <i>Uropterygius</i> sp.	Labridae	<i>Bodianus mesothorax</i> <i>Diproctacanthus xanthurus</i> <i>Halichoeres melanurus</i> <i>Halichoeres purpurascens</i> <i>Pseudocheilinus hexataenia</i>
Ophichthidae	<i>Muraenichthys macropterus</i> <i>Ophichthus</i> sp.	Opistognathidae	<i>Opistognathus reticulatus</i>
Clupeidae	<i>Dussumieria elopsoides</i>	Pholidichthyidae	<i>Pholidichthys anguis</i>
Engraulidae	<i>Encrasicholina devisi</i> <i>Stolephorus indicus</i> <i>Stolephorus</i> sp.	Blenniidae	<i>Cirripectes alleni</i> <i>Ecsenius lividinalis</i> <i>Omobranchus ferox</i> <i>Omobranchus lineolatus</i>
Plotosidae	<i>Paraplotosus muelleri</i>	Gobiidae	<i>Amblyeleotris gymnocephalus</i> <i>Amblygobius nocturnus</i> <i>Amoya madraspatensis</i> <i>Boleophthalmus caeruleomaculatus</i> <i>Butis amboinensis</i> <i>Cryptocentroides insignis</i> <i>Cryptocentrus cinctus</i> <i>Cryptocentrus strigilliceps</i> <i>Drombus triangularis</i> <i>Favonigobius melanobranchus</i> <i>Glossogobius circumspectus</i> <i>Gobiopsis aporia</i> <i>Macrodonogobius wilburi</i> <i>Pandaka lidwilli</i> <i>Paragobiodon xanthosomus</i> <i>Pleurosicya fringilla</i> <i>Prionobutis microps</i> <i>Parioglossus palustris</i> <i>Parioglossus philippinus</i>
Hemirhamphidae	<i>Rhynchorhamphus georgii</i> <i>Zenarchopterus gilli</i>		<i>Siganus vulpinus</i>
Platycephalidae	<i>Papilloculiceps bosschei</i>	Soleidae	<i>Aseraggodes klunzingeri</i> <i>Rendahlia jaubertensis</i>
Serranidae	<i>Cephalopholis leopardus</i> <i>Epinephelus ongus</i>	Tetraodontidae	<i>Arothron nigropunctatus</i> <i>Chelonodon patoca</i> <i>Marilyna darwinii</i>
Pseudochromidae	<i>Assiculoides desmonotus</i> <i>Labracinus cyclophthalmus</i>	Diodontidae	<i>Cyclichthys orbicularis</i>
Notograptiidae	<i>Notograptus</i> sp.		
Apogonidae	<i>Apogon lateralis</i> <i>Apogon unicolor</i> <i>Gymnapogon urospilotus</i> <i>Pseudamia nigra</i>		
Leiognathidae	<i>Gazza achlamys</i>		
Lutjanidae	<i>Lutjanus johnii</i>		
Haemulidae	<i>Plectorhinchus gibbosus</i>		
Chaetodontidae	<i>Chaetodon vagabundus</i> <i>Chelmon muelleri</i>		
Pomacanthidae	<i>Chaetodontoplus mesoleucus</i>		
Pomacentridae	<i>Acanthochromis polyacanthus</i> <i>Amblyglyphidodon batunai</i> <i>Amphiprion ocellaris</i> <i>Chrysiptera rollandi</i> <i>Dischistodus darwiniensis</i> <i>Hemiglyphidodon plagiometopon</i> <i>Pomacentrus adelus</i> <i>Pomacentrus littoralis</i>		

The fishes inhabiting the south-western portion are a continuation of the fauna found along the Pilbara coast to the south, i.e., from Exmouth Gulf to near Broome. This zone is characterized by an arid to semi-arid climate and has few large rivers flowing into the sea. Numerous species occur no further north than Cape Leveque, whereas others that are present in moderate numbers south of Cape Leveque were found only in small numbers to the north (Table IV). This suggests that some environmental conditions in the Cape Leveque area are preventing these species from ranging further northwards. One barrier to dispersal would appear to be water quality as turbidity increases greatly in the King Sound area just to the north of Cape Leveque. In addition, a coastal morphological zone boundary (Wright, 1964; Brooke, 1995) and botanical zone boundary (Semeniuk *et al.*, 1978; Beard, 1990) are known to exist at Cape Leveque.

The North-east Kimberley includes Cambridge Gulf with its large river deltas. It has a moderate to heavy rainfall during the summer causing a heavy outflow from its rivers. The waters in Cambridge Gulf are exceptionally turbid, a condition which extends north-west along the coast. Furthermore, there are two large rivers to the west, the King George and the Berkley, which contribute to this turbidity. This region possesses fish species that are more typical of the Northern Territory (Table V). Some of these range at least to the mouth of the King George River but are absent from areas further to the west. For example, intertidal rock pools in the North-east Kimberley contain the chaetodontid *Chelmon muelleri*, whereas those in the North and South-west Kimberley contain *C. marginalis*. In addition, only one endemic Western Australian species - *Acanthopagrus palmaris* - was recorded to the east of the mouth of the King George River, and this was on the basis of only a single specimen. A coastal morphological zone boundary (Brooke, 1995) and apparent botanical boundary (Semeniuk *et al.*, 1978) exist in the region of

Table IV. - Species not or rarely recorded north of Cape Leveque.

Family	Species	Family	Species
Serranidae	<i>Epinephelus bilobatus</i>	Sphyraenidae	<i>Sphyraena obusata</i>
	<i>Epinephelus multinotatus</i>	Labridae	<i>Choerodon cauteroma</i>
Grammistidae	<i>Diploprion bifasciatum</i>		<i>Coris aygula</i>
Pseudochromidae	<i>Assiculus punctatus</i>		<i>Coris caudimacula</i>
	<i>Labracinus lineatus</i>		<i>Coris pictoides</i>
Apogonidae	<i>Apogon cavitiensis</i>	Trypterygiidae	<i>Helcogramma striatus</i>
Lethrinidae	<i>Lethrinus</i> sp.	Blenniidae	<i>Petroscirtes breviceps</i>
Nemipteridae	<i>Pentapodus emeryii</i>	Gobiidae	<i>Valenciennesa alleni</i>
	<i>Scolopsis taeniopterus</i>	Acanthuridae	<i>Ctenochaetus striatus</i>
Kyphosidae	<i>Kyphosus vaigiensis</i>	Monacanthidae	<i>Colurodonotus paxmani</i>
Chaetodontidae	<i>Coradion chrysozonus</i>	Ostraciidae	<i>Rhynchostracion rhinorhynchus</i>
Pomacanthidae	<i>Chaetodontoplus personifer</i>	Tetraodontidae	<i>Arothron stellatus</i>
Pomacentridae	<i>Pomacentrus nagasakiensis</i>	Diodontidae	<i>Diodon liturosus</i>

Family	Species
Hemiramphidae	<i>Rhynchorhamphus georgii</i>
Apogonidae	<i>Apogon lateralis</i>
	<i>Pseudanania nigra</i>
Chaetodontidae	<i>Chelmon muelleri</i>
Tetraodontidae	<i>Chelonodon patoca</i>

Table V. - Species not or rarely recorded to the west of the King George River.

Cape Rulhieres near the King George River mouth, so this area may also represent a barrier to dispersal for some species of fish. However, only a small number of fish surveys have been done along this portion of the coastline, so insufficient information is currently available to test this hypothesis.

The North Kimberley receives the highest rainfall of the three zones (up to 1,600 mm per wet season) and also has waters with some of the highest turbidity. However, some of its outer islands, particularly in the Bonaparte Archipelago (Fig. 1), are surrounded by waters of low to moderate turbidity only. Its coastal morphology is also diverse, and for this reason attracted most of the survey activity (430 species recorded) during the present study. The individual sites with the highest diversities were in the outer islands where up to 75 species per dive were recorded. However, one mainland site near Cape Londonderry - the northernmost point of mainland Western Australia - produced 72 species which was exceptional (inshore surveys usually produced totals of only 20-30 species per site). Table VI lists some of the species found commonly in this zone but not or rarely in the other two. A number of the species recorded from the outer islands were transients from the offshore atolls, and were present in low numbers only.

In order to compare the fishes of the Kimberley with those of other regions of Western Australia's northern half, the following analysis concentrates on the composition of four families, the Chaetodontidae, the Pomacentridae, the Labridae, and the Acanthuridae. These families were selected because each is well known taxonomically, the distributions of their species in Western Australia have been extensively documented, and all are readily visible to divers. An examination of their diversity in each region is a good

Table VI. - Species commonly recorded in the North Kimberley, but not or rarely found in the South-west and North-east Kimberley.

Family	Species	Family	Species
Muraenidae	<i>Gymnothorax</i> sp.	Pomacentridae	<i>Acanthochromis polyacanthus</i>
Plotosidae	<i>Paraplotosus muelleri</i>		<i>Amblyglyphidodon batunai</i>
Serranidae	<i>Epinephelus ongus</i>		<i>Pomacentrus adelus</i>
Pseudochromidae	<i>Assiculoides desmonotus</i>	Labridae	<i>Halichoeres melanurus</i>
	<i>Labracinus cylophthalmus</i>	Opistognathidae	<i>Opistognathus reticulatus</i>
Notograptiidae	<i>Notograpus</i> sp.	Blenniidae	<i>Cirripectes alleni</i>
Pomacanthidae	<i>Chaetodontoplus mesoleucus</i>		

Table VII. - Numbers of tropical species of four families occurring in various regions of northern Western Australia (abbreviations: Ningal - Ningaloo Reef; Monteb - Monte Bello Islands; Damp - Dampier Archipelago; Kimb - Kimberley; Rowley - Rowley Shoals; Scott/S - Scott and Seringapatam Reefs; Ashmor - Ashmore Reef; N WA - northern Western Australia) (sources: Allen, 1991, 1992, 1993a, 1993b, 1996; Allen and Hutchins, unpubl. data; Allen and Russell, 1986; Hutchins, 1978, 1994, 1995, 1996a, 1996b; Hutchins *et al.*, 1995).

	Ningal	Monteb	Damp	Kimb	Rowley	Scott/S	Ashmor	N WA
Chaetodontidae	24	17	18	13	25	31	30	42
Pomacentridae	40	37	32	31	49	60	72	93
Labridae	59	37	40	34	58	63	59	100
Acanthuridae	22	11	9	6	20	23	27	32
Totals	145	102	99	84	152	177	188	267

indicator to the richness of other families there, especially those that are not as well known. This in turn can be used to produce a reasonably simple method to quickly assess the relative diversity of the fishes inhabiting each region (Done *et al.*, 1994).

Table VII shows the number of tropical species in each family in seven regions of Western Australia. The last column gives the total number of species known for each family in the northern half of Western Australia (excluding trawl-caught and deepwater species). This comparison indicates that the lowest diversity occurs in the nearshore waters of the Kimberley, followed by the Dampier Archipelago and the Monte Bello Islands. Ningaloo Reef and the Rowley shoals are similar in species numbers, being only a little lower than Scott and Seringapatam Reefs, with Ashmore Reef having the highest diversity. The regions possessing high diversities all occur in areas of good water visibility while those with low diversities are located in areas with turbid waters. One exception is the offshore Monte Bello Islands which has a fish fauna more similar to that of the inshore Dampier Archipelago. Perhaps the location of the Monte Bello Islands well to the east of the 200 m contour line (Fig. 1) indicates that conditions there are less oceanic than at Ningaloo Reef which is located on the mainland coast but close to this depth contour. Certainly the turbidity of the waters surrounding the Monte Bello Islands can increase dramatically following a cyclonic weather disturbance (Hutchins, pers. obs.). The effect of a heavy outflow of silt-laden waters from the many rivers along the Pilbara coast can be seen many kilometres out to sea. Nevertheless, the Monte Bello Islands and Dampier Archipelago generally have less turbid waters than the Kimberley, and as a direct result, have comparatively higher fish diversities.

The ten most abundant reef species, based on visual surveys, for the nearshore Kimberley are shown in table VIII, together with the ten most abundant from the Dampier Archipelago, Ningaloo Reef and the offshore atolls for comparison. The Kimberley and Dampier Archipelago species lists are almost identical, the most notable difference being the pomacentrid *Acanthochromis polyacanthus* which does not occur to the south of the Kimberley. As expected, the list for the offshore atolls generally has little in common with those from either the nearshore Kimberley or Ningaloo Reef. This suggests that the turbid water fauna along the coastline of Western Australia's northern half is more consistent in species composition than the fauna of non-turbid water areas. Furthermore, at least five of the Kimberley and Dampier Archipelago species are also abundant in inshore wa-

Table VIII. - Ten most abundant species at four areas in northern Western Australia (listed in decreasing order of abundance).

Kimberley	Dampier Archipelago	Ningaloo Reef	Offshore Atolls
<i>Abudefduf bengalensis</i>	<i>Pomacentrus milleri</i>	<i>Thalassoma lunare</i>	<i>Dascyllus aruanus</i>
<i>Pomacentrus milleri</i>	<i>Neopomacentrus azyron</i>	<i>Thalassoma lutescens</i>	<i>Pomacentrus adelus</i>
<i>Choerodon cyanodus</i>	<i>Halichoeres nigrescens</i>	<i>Chromis atripectoralis</i>	<i>Amblyglyphidodon curacao</i>
<i>Acanthurus graminoptilus</i>	<i>Abudefduf bengalensis</i>	<i>Pomacentrus vaiuli</i>	<i>Ctenochaetus striatus</i>
<i>Lutjanus carponotatus</i>	<i>Thalassoma lunare</i>	<i>Pomacentrus moluccensis</i>	<i>Chromis viridis</i>
<i>Halichoeres nigrescens</i>	<i>Chaetodon aureofasciatus</i>	<i>Abudefduf sexfasciatus</i>	<i>Chrysiptera hemicyanea</i>
<i>Dischistodus darwiniensis</i>	<i>Lutjanus carponotatus</i>	<i>Pomacentrus coelestis</i>	<i>Chromis ternatensis</i>
<i>Neopomacentrus azyron</i>	<i>Acanthurus graminoptilus</i>	<i>Pomacentrus milleri</i>	<i>Pomacentrus lepidogenys</i>
<i>Acanthochromis polyacanthus</i>	<i>Caesio cuning</i>	<i>Scarus sordidus</i>	<i>Scarus sordidus</i>
<i>Chaetodon aureofasciatus</i>	<i>Choerodon cyanodus</i>	<i>Plectroglyphidodon lacrymatus</i>	<i>Hippocampus longiceps</i>

ters of Queensland. Finally, not one Western Australian endemic is included in these lists of most abundant species (by contrast, endemic species in subtropical and temperate waters of Western Australia comprise the majority of the most often sighted species, see Hutchins, 1994).

Table IX presents a comparison of offshore and inshore fish numbers for the four families mentioned above between northern Western Australia and Queensland. Published species lists for nearshore waters of Queensland were not available, but the results of numerous years of sampling at Orpheus Is. by James Cook University (Bellwood, unpubl. data) show some similarity in species numbers to those of the Kimberley. Orpheus Is., which is located a few kilometres off the Queensland coast just to the north of Townsville, is at about the same latitude as Broome in the southern Kimberley. Species diversity at Orpheus Island is comparatively low due to the moderate turbidity of the surrounding waters. The offshore Coral Sea islands have a much higher species diversity which is comparable to those at the offshore atolls in Western Australia. Interestingly, total species numbers for north-western reefs of Western Australia and those of the Great Barrier Reef also show little difference. The unexpected richness of the former reefs is probably due to the close proximity of the rich Indonesian faunal region.

## CONCLUSION

In conclusion, the nearshore marine fish fauna of the Kimberley, which at present totals 469 species, is apparently typical of other turbid water areas of northern Australia. A proportion of its fauna (3%) is endemic to Western Australia which provides a small but distinctive component (only 1% is endemic to the Kimberley). Whereas the south-western portion of the Kimberley supports species more typical of the Pilbara region to the south, the north-eastern portion is inhabited by species apparently more representative of the Northern Territory. The northern portion contains a majority of species that typify the Kimberley. These preliminary findings suggests that the Kimberley should be divided into three faunal zones, a south-western, a northern and a north-eastern one.

Table IX. - Numbers of tropical species of four families occurring in various areas of north-western and north-eastern Australia (abbreviation: Rowley, Scott, Ash - Rowley Shoals, Scott and Seringapatam Reefs, and Ashmore Reef) (sources: Allen, 1988; Randall *et al.*, 1990; Bellwood, unpubl. data; see also references listed for table VII).

	Western Australia			Queensland		
	North-western reefs	Rowley, Scott, Ash	Kimberley	Orpheus Island	Coral Sea islands	Great Barrier Reef
<b>Chaetodontidae</b>	42	36	13	17	31	43
<b>Pomacentridae</b>	93	78	31	38	67	92
<b>Labridae</b>	100	78	34	33	70	105
<b>Acanthuridae</b>	32	29	6	5	31	33
<b>Totals</b>	267	221	84	93	199	273



**Acknowledgements.** - I wish to thank my co-workers on the Kimberley fish surveys, G.R. Allen and S.M. Morrison for allowing me to use their data. I would also like to acknowledge one of the organisers, F.E. Wells (Western Australian Museum), for inviting me to participate in this study. I am particularly grateful to the following specialists for help with identifications: G.R. Allen, Western Australian Museum (Apogonidae and Pomacentridae), A.C. Gill, Natural History Museum (Pseudochromidae), and H. Larson, Northern Territory Museum (Gobiidae, Microdesmidae and Pholidichthyidae). D.R. Bellwood, James Cook University, kindly sent me portions of a preliminary fish list for Orpheus Island, while A. Thompson, Australian Institute of Marine Science, made available information on the fishes of northern Queensland. Finally S.M. Morrison provided much help with the onerous task of identification and curation of the Kimberley collections.

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## THE STRUCTURE OF THE OUTER COMPONENTS OF THE SCALES OF *LATIMERIA CHALUMNAE* (SARCOPTERYGII: ACTINISTIA: COELACANTHIDAE) REVISITED USING SCANNING AND TRANSMISSION ELECTRON MICROSCOPY

by

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**ABSTRACT.** - SEM (scanning electron microscopy) and TEM (transmission electron microscopy) studies confirmed that the scales of *Latimeria chalumnae* can be considered as elasmoid scales because of their organization. The external layer is made of thin collagen fibrils (about 30 nm in diameter) organized in a loose network which contains an abundant extrafibrillar matrix rich in mucosubstances. Its ultrastructural aspect resembles that of the external layer of teleost elasmoid scales. Globular corpuscles are found in the isopedine closest to the external layer. Their mineralization is isotropic as in the Mandl's corpuscles of teleost elasmoid scales. The odontodes set on the outer ridges are made of mineralized tissues similar to those described in the teeth of the coelacanth. The ultrastructural study of *Latimeria* scales revealed the presence of mineralized tissues the structure of which is similar to that found in the teleostean elasmoid scales and is considered as a specific characteristic of this type of scale.

**RÉSUMÉ.** - La structure des composants superficiels des écailles de *Latimeria chalumnae* (Sarcopterygii: Actinistia: Coelacanthidae) revue grâce au microscope électronique à balayage et à transmission.

L'organisation générale des écailles de *Latimeria chalumnae* avait conduit à assimiler ces dernières à des écailles élasmoïdes, ce que confirme notre étude ultrastructurale. En surface, une couche limitante externe n'a pas pu être mise en évidence, mais la couche externe présente un aspect ultrastructural semblable à celui des écailles élasmoïdes des Téléostéens. Elle est constituée par une trame lâche de fines fibrilles de collagène (d'environ 30 nm de diamètre) entre lesquelles se trouve une matrice extrafibrillaire riche en mucosubstances. Des corpuscules minéralisés sphériques sont observés dans l'isopédine, à la limite de la couche externe. Leur minéralisation est isotropique comme pour les corpuscules de Mandl des écailles élasmoïdes des Téléostéens. Les odontodes qui sont disposés à la surface des crêtes externes sont constitués des mêmes tissus minéralisés que ceux qui ont été décrits pour les dents du Coelacanth. L'analyse ultrastructurale révèle donc la présence dans les écailles élasmoïdes de *Latimeria* de tissus minéralisés dont la structure est celle qui a été observée dans les écailles élasmoïdes des Téléostéens.

**Key-words.** - Coelacanthidae, *Latimeria chalumnae*, Scales, External layer, Odontodes, SEM, TEM.

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The coelacanth (*Latimeria chalumnae*), the only extant fish of the important group of Paleozoic "Crossopterygii", shows a body covered with numerous large imbricated scales (Millot and Anthony, 1958; Locket, 1980) which agree with the concept of elasmoid scales (Smith *et al.*, 1972; Castanet *et al.*, 1975; Meunier, 1980; 1984) as defined by Bertin (1944) in the Teleostei. Indeed, the scales of the coelacanth are 1) thin and flexible plates; 2) imbricated, their anterior part is deeply inserted within the dermis and their posterior part overlaps the lateral and the anterior fields of the neighbouring scales; 3) composed of two parts: a thin mineralized ornamented superficial layer topping a thick basal plate made of cellular isopedine which is almost entirely unmineralized. In the coelacanth, the isopedine is made of thick collagen fibrils (from 100 to 130 nm in diameter) (Castanet *et al.*, 1975; Giraud *et al.*, 1978a; Meunier, 1980). The collagen fibrils form bundles (Giraud *et al.*, 1978a) which are arranged in a twisted plywood fashion and the progressive rotation of the fibril directions is right-handed (Giraud *et al.*, 1978a, 1978b). However, the coelacanth elasmoid scale differs from that of the Teleostei within which isopedine does not show collagen fibrils arranged in bundles and the rotation direction is left-handed (Meunier and Castanet, 1982; Meunier, 1987-88). Moreover, the presence of numerous odontodes scattered on the radial ridges of the posterior field (Smith *et al.*, 1972; Castanet *et al.*, 1975; Smith, 1979) is characteristic of the coelacanth scales since, up to now, odontodes or similar superficial ornamentations were not found on the surface of teleostean elasmoid scales except on the scales of *Denticeps clupeioides*, a clupeomorph fish (Sire *et al.*, 1998).

Coelacanthids and Teleostei belong to two different osteichthyan lineages, respectively Sarcopterygii and Actinopterygii. Sarcopterygii were characterized by the presence of cosmoid scales which are known only in fossils whereas ganoid scales, characteristic of primitive Actinopterygii, are found not only in extinct representatives of this group but also in extant Polypteridae and Lepisosteidae (Goodrich, 1907; Schultze, 1977; Janvier, 1996). Although these two primitive types of scales have evolved apart, nevertheless, they have reached a similar derived (elasmoid) state respectively in Coelacanth + Dipnoi (Castanet *et al.*, 1975; Meunier and François, 1980) and Teleostei (Sire and Meunier, 1981; Meunier, 1984; Meunier, 1997, *inter alia*). The elasmoid scale is characterized by a reduction of thickness and mineralization (Schultze, 1977, 1996) and by the presence of peculiar mineralized tissues which differ from typical bony tissues of the endoskeleton (Zylberberg *et al.*, 1992). Ultrastructural analyses of the mineralized tissues were carried out on the ganoid scales of extant Actinopterygii (Sire *et al.*, 1987; Sire, 1989, 1990, 1994) and on elasmoid scales of Teleostei (review in Zylberberg *et al.*, 1992). Ultrastructural studies on the coelacanth scales concern the organization of the isopedine (Giraud *et al.*, 1978a, 1978b) but a few ultrastructural data are available to characterize accurately the tissues constituting the superficial layers of the scale and of the odontodes (Smith *et al.*, 1972). The present report on the ultrastructural organization of the external layer and of the odontodes of *Latimeria chalumnae* scales lead to an examination of possible homologies between the components of elasmoid scales in both taxa: the Coelacanthidae and the Teleostei.

## MATERIAL AND METHODS

### Material

Several scales overlaying the basis of the pelvic fin of *Latimeria chalumnae* (female N°67, 78 kg, 163 cm) have been fixed in neutral formalin and kept in ethanol 70° for light microscopic studies. The formalin fixation which ensure a structural preservation allowing SEM and even TEM observations was achieved by treatments usually used for SEM and TEM (see below).

### Methods

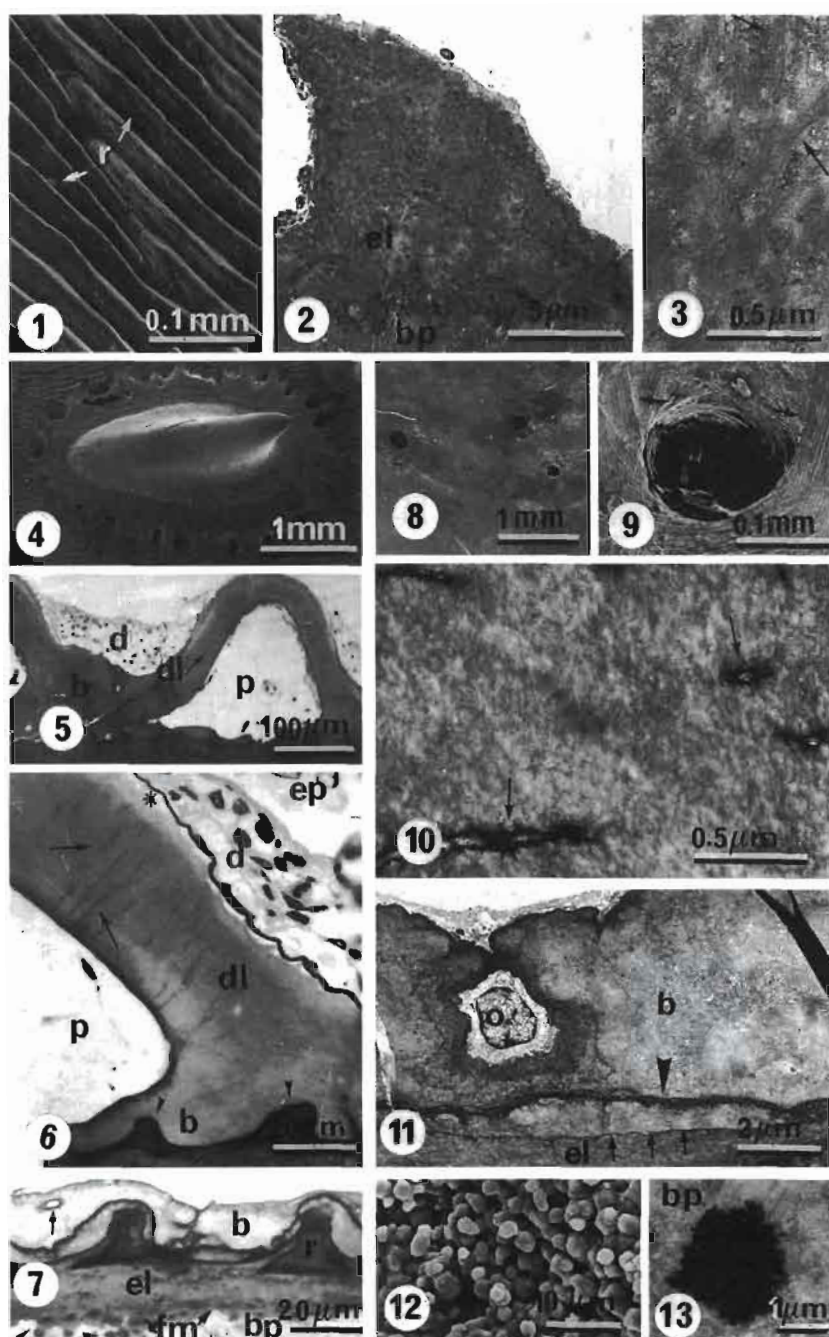
For the SEM observations, the scales were steeped in 6 or 12% sodium hypochloride solution at room temperature to strip the surface of the scales, to destroy the unmineralized collagenous fibers and to clean the mineralizing front of the basal plate. Then the scales were washed in distilled water, dehydrated in absolute ethanol, air-dried, glued and coated with evaporated gold. The upper and deep surfaces of the scales were examined in a JEOL-SEM-35 scanning electron microscope.

For TEM, small pieces from the anterior and posterior fields of scales were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer pH 7.4. Some samples were decalcified using 0.1 M EDTA added to the fixative solution. All the samples were placed in 1% osmium tetroxide in the cacodylate buffer, then dehydrated and embedded in Epon. Thick sections (thickness  $\approx 1 \mu\text{m}$ ) were stained with buffered toluidine blue solution for light microscopic examination. Ultrathin sections of selected areas were double stained with uranyl acetate and lead citrate and viewed at 80 kV in a Philips 201 transmission electron microscope.

## RESULTS

SEM examination shows that the whole outer surface of the scale is ornamented with radial ridges. These ridges are present on the surface of the anterior field (Fig. 1) which is inserted within the dermis and covered by the neighbouring scales as well as on the surface of the uncovered posterior field where odontodes are located. The TEM examination of vertical sections of the ridges in the anterior field revealed that they are constituted by elevations of the external layer (Fig. 2). This layer contains thin collagen fibrils (about 30 nm in diameter) organized in a loose network (Fig. 3) within which electron-opaque granules could represent mucosubstances involved in the nucleation of the mineral deposit. At the scale margin, the external layer is first deposited and the basal plate is subsequently formed as in the teleostean elasmoid scales (see Sire and Géraudie, 1983).

The odontodes are restricted to the posterior field of the scales and are isolated (Fig. 4) or associated to form odonto-complexes (Fig. 5). An isolated odontode is composed of two parts: a spiny denticle made of dental tissues and overlying a star-like basis which is made of a bony tissue; it is in close contact with the ridges of the scale surface (Figs 5-7). Holes scattered on the surface of the bony basis are the openings of vascular canals crossing this bony tissue (Fig. 4). A vascular network is associated with the odonto-complexes; it is composed of vascular cavities and canals which are located in the bony basis of the odontode. Some vascular canals cross the inner part of the scale and open on the innermost surface of the basal plate in the posterior part of the scale (Figs 8, 9).



The top of the odontodes is covered by a thin layer of enamel and it is partially covered by the epidermis (Fig. 6). The odontodes are surrounded by the loose superficial dermis (Figs 5, 6). A thick basement membrane separates the epidermis from the dermis. Orthodontine surrounds the pulp cavity (Figs 5, 6) and dentine with a globular aspect is observed in the basal part of the odontode in contact with the bony base. Ultrastructural observations show parallel odontoblastic canaliculi in the circumpulpar dentine (Fig. 10).

The bony basal star-like pad anchors the odontode to the underlying external layer (Figs 4, 6). This tissue shows a lamellar or a pseudolamellar organization. A thin cementing line is present between this bony tissue and the underlying external layer (Fig. 11) and between the older odontodes and the more superficial new-formed ones which form the odonto-complexes. Thick cementing lines are also observed within the bony tissue where they show a laminate aspect (Fig. 11). All these cementing lines look like those described in normal bone (Castanet, 1981).

The basal plate is composed of thick collagen fibrils as previously described elsewhere (Giraud *et al.*, 1978b). It is almost entirely unmineralized. Mineral is only deposited within a thin layer close to the external layer. There the mineralization front is ornamented with numerous spherical mineralized corpuscles (Figs 7, 12). These 1-2  $\mu\text{m}$  width corpuscles are located ahead of the mineralizing front (Fig. 7). Their mineralization is isotropic (Fig. 13) like the mineralization of the external layer. The corpuscles become imbedded within the mineralized part of the basal plate when the mineral deposit invades the deeper parts of the isopedine.

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Figs 1-3. - Anterior field of a scale of *Latimeria chalumnae*.

Fig. 1. - SEM. The anterior field is ornamented with parallel ridges (r) which are radially oriented. Fig. 2. - TEM. Vertical section of the scale showing a ridge transversally sectionned. The ridge is an elevation of the external layer (el) which covers the basal plate (bp). Fig. 3. - TEM. Detail of external layer showing the loose arrangement of the thin collagen fibrils (arrows) within the extracellular matrix.

Figs 4-13. - Posterior field ornamented with odontodes in the scale of *Latimeria chalumnae*.

Fig. 4. - SEM. An isolated odontode located at the scale surface. The central sharp-pointed part of the odontode shows dental tissues (details in Figs 5, 6, 8) and the marginal flat part is made of a bony tissue (see Fig. 7). Holes (arrows) indicate the openings of vascular canals. Fig. 5. - Semi-thin section stained with buffered toluidine blue. Vertical section of odontodes showing the central pulp cavity (p) limited by a layer of dentine (dl) next to bone (b) and surrounded by the dermis (d). Fig. 6. - Semi-thin section stained with buffered toluidine blue. Detail of an odontode showing the pulp cavity surrounded by the orthodontine (dl) crossed by cell processes (arrows). At the outer surface, a thin layer of enamel appears a light space (\*). The dermis (d) and epidermis (ep) cover the outer surface of the odontode. The odontode lie on the small ridges (arrowheads) of the outer surface of the scale. b: bony tissue. Fig. 7. - Semi-thin section stained with buffered toluidine blue. Detail of the margin of an odontode. The bony tissue (b) contains osteocyte lacunae (arrows). It covers the small ridges of the outer surface of the scale (r). Mandl's corpuscles (arrowheads) are forward of the mineralization front (fm) which is located in the basal plate in the vicinity of the external layer. Fig. 8. - SEM. Deep surface of the posterior area of the scale showing the openings of vascular canals. Fig. 9. - SEM. Detail of an opening in the same area showing the helicoidal arrangement of bundles of collagen fibrils. Fig. 10. - TEM. Section through the dentine layer crossed by cell processes (arrows). Fig. 11. - TEM. Section through the bony basis (b) of the odontode. The bony tissue which contains osteocytes (o) shows a thick cementing line (arrowhead) and it is separated from the external layer (el) by a thin cementing line (arrows). Fig. 12. - SEM. The mineralizing front is ornamented with abundant Mandl's corpuscles. Fig. 13. - TEM. Section of a Mandl's corpuscle within the basal plate (bp) showing the mineral deposited in the collagen fibrils.

## DISCUSSION

The present ultrastructural data support the contention that the scales of the extant sarcopterygian fish *Latimeria chalumnae* exhibit features similar to those of the elasmoid scales of the Teleostei which belong to the Actinopterygian lineage. The general organization of the coelacanth scales was already considered to be consistent with the view of a basic structural organization in the elasmoid scales which consist of a basal plate made of a more or less mineralized collagen network (= isopedine of Meunier, 1984) arranged in a regular plywood-like structure and a well-mineralized superficial layer composed of heterogeneous mineralized tissues (Zylberberg *et al.*, 1992). The mineralized corpuscles of the isopedine can be considered as Mandl's corpuscles even if, in *Latimeria*, they keep a spherical shape contrary to the various polymorphic shapes of Mandl's corpuscles in Teleostei (Schönborn *et al.*, 1981; Zylberberg *et al.*, 1992).

The radial ridges ornamenting the whole surface of the scales are made of a mineralized tissue which shows a structure similar to that described in the external layer of the elasmoid scale in Teleostei (Zylberberg and Nicolas, 1982; Sire, 1985). In *Latimeria*, the external layer is probably the first layer deposited during the ontogenic development of the scale as in the teleost elasmoid scales (Maekawa and Yamada, 1970; Sire and Géraudie, 1983; Sire *et al.*, 1997). The present ultrastructural analysis supports the hypothesis that both external layers could be homologous in the elasmoid scales of *Latimeria* and Teleostei.

On the posterior field of the coelacanth scales, the odontodes lie on the external layer as already shown by Smith *et al.* (1972). The surface of the scales do not show any outer layer which could be compared to the outer limiting layer described in the elasmoid scale of the Teleostei (Schönborn *et al.*, 1979; Sire, 1985), and characterized by the lacking of collagen fibrils and the abundance of mucosubstances (Zylberberg and Nicolas, 1982). An outer limiting layer similar to that of the teleostean elasmoid scales was not found in the dipnoan scales (Zylberberg, 1988) which are also considered as elasmoid scales (Meunier and François, 1980). It is noteworthy that the elasmoid scales of the extant Sarcopterygii (*Protopterus* and *Latimeria*) lack a layer similar to the outer limiting layer of the teleostean scales. The outer limiting layer could be a specific structure restricted to the actinopterygian lineage.

The odontodes of the posterior field of *Latimeria* scales appear as poorly developed homologs of the extensive odonto-complexes that constituted the cosmine of cosmoid scales (Ørvig, 1968, 1969, 1977; Thomson, 1975). They contribute to the thickening of the posterior part of the scale and their sharp head could facilitate the anchoring of the scale within the integument. It could be hypothesized that in the actinopterygian lineage, the absence of odontodes in the great majority of teleosts has been balanced by the development of specific structures such as various ornamentations constituted by the outer limiting layer and/or the ctenii in ctenoid scales (see Roberts, 1993) which contribute to thicken the posterior field and to anchor the scale within the integument.

**Aknowledgements.** - We are grateful to Françoise Allizard and Christiane Chancogne for their technical assistance, respectively for TEM and SEM.



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## HISTOLOGICAL STRUCTURE OF THE CAUDAL SPINE OF THE SURGEONFISH *CTENOCHAETUS STRIATUS* (TELEOSTEI: ACANTHURIDAE)

by

François J. MEUNIER (1, 2) & Hélène FRANCILLON-VIEILLOT (2)

**ABSTRACT.** - The caudal spines (=“scalpel-like spines”) of the surgeonfish are considered to be specialized scales. In *Ctenochaetus striatus* caudal spines consist of acellular bone. They are divided in two parts: a central one, the medulla, made of spongy bone; it is surrounded by the second part made up of cortical primary avascular bone. This cortical bone shows concentric growth marks that indicate an appositional growth. Medullar bone is clearly less mineralized than cortical bone and numerous cementing lines can be seen. This means that the medulla is the result of a remodeling phenomenon of primary bone. The relationships between the components of the caudal spine and those of scales are discussed.

**RÉSUMÉ.** - Structure histologique des épines caudales chez le poisson-chirurgien *Ctenochaetus striatus* (Teleostei: Acanthuridae).

Les épines caudales (=“lancettes”) des poissons chirurgiens sont considérées comme des écailles spécialisées. Chez *Ctenochaetus striatus*, ces épines sont constituées d’os acellulaire. Le corps de l’épine se divise en deux parties: une zone centrale, la médulla, constituée d’os spongieux; elle est entourée par la seconde, un cortex fait d’un os primaire avasculaire. Cet os cortical présente des marques de croissance concentriques ce qui indique une croissance appositionnelle. L’os médullaire, riche en lignes cimentantes, est nettement moins minéralisé que l’os cortical. Ceci indique que la médulla est le résultat d’un processus de remaniement de l’os primaire. Les relations entre les différents composants de l’épine et ceux d’une écaille sont discutées.

**Key-words.** - Acanthuridae, *Ctenochaetus striatus*, Caudal spine, Histology, Bone.

Acanthuridae or surgeonfishes are tropical marine fishes that live in shallow waters surrounding coral reefs. They are characterized by one to several caudal spines which are considered as modified scales by the authors. In *Acanthurus* and *Ctenochaetus*, which are sister groups (Winterbottom, 1993; Winterbottom and McLennan, 1993), there is only one sharp, movable scalpel-like caudal spine (Fig. 1), anchored on each side of the caudal peduncle (Guigas and Winterbottom, 1993). The sharp apical extremity is anteriorly orientated and can provoke serious injuries (or wound) (Randall, 1959; Winterbottom, 1971). The basal part of this caudal spine is attached to the subjacent vertebra by a ligament (Fig. 2; Winterbottom, 1971). In *Ctenochaetus* (as in *Acanthurus*) the spiny scale folds back into a deep groove in the skin (Souché, 1935; Winterbottom, 1971). There are no muscles to move the caudal spines. In fact, both spines would be alternately

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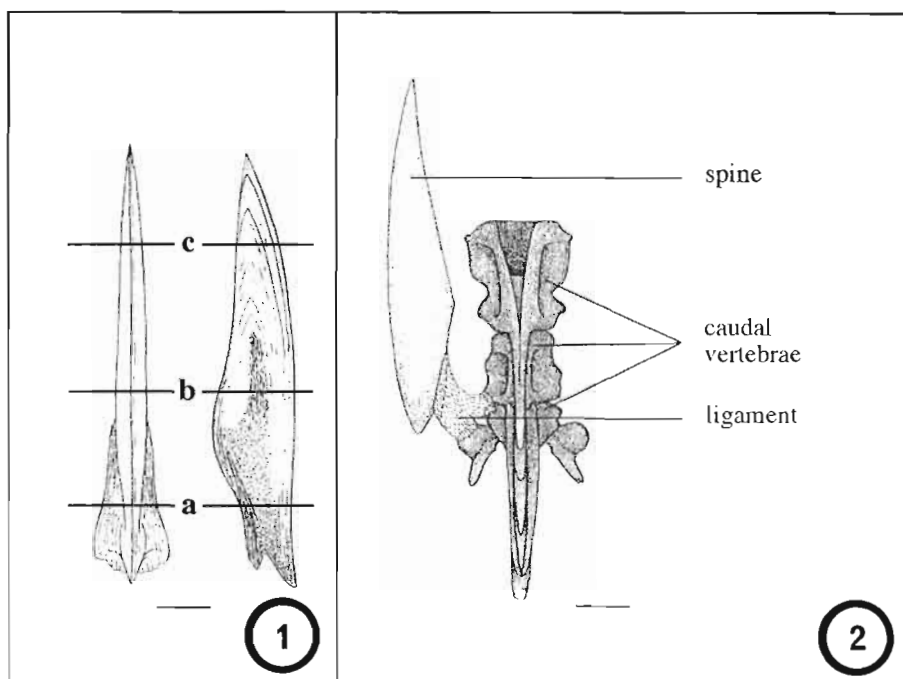


Fig. 1. - Lateral and dorsal views of the caudal spine of *Acanthurus monroviae* (after Monod, 1959). a, b and c localize approximately the transverse sections of figures 3 to 5. Bar = 2 mm.

Fig. 2. - Articulation of the left spine and vertebra of *Acanthurus lineatus* (dorsal view) (after Winterbottom, 1971). Bar = 2 mm.

exposed for each oscillation of the caudal peduncle during swimming (Winterbottom, 1971). According to Monod (1959), the caudal spine has the same constitution as a scale, more precisely as the homogeneous distal layer, in other words the external layer. Nevertheless, the skeletal tissue of the proximal half of the spine has a spongy texture (Monod, 1959) with numerous canals and/or cavities. This kind of structure is uncommon in elasmoid scales. According to Monod (1959), the membranous groove which contains the spine could be considered as the scale pocket.

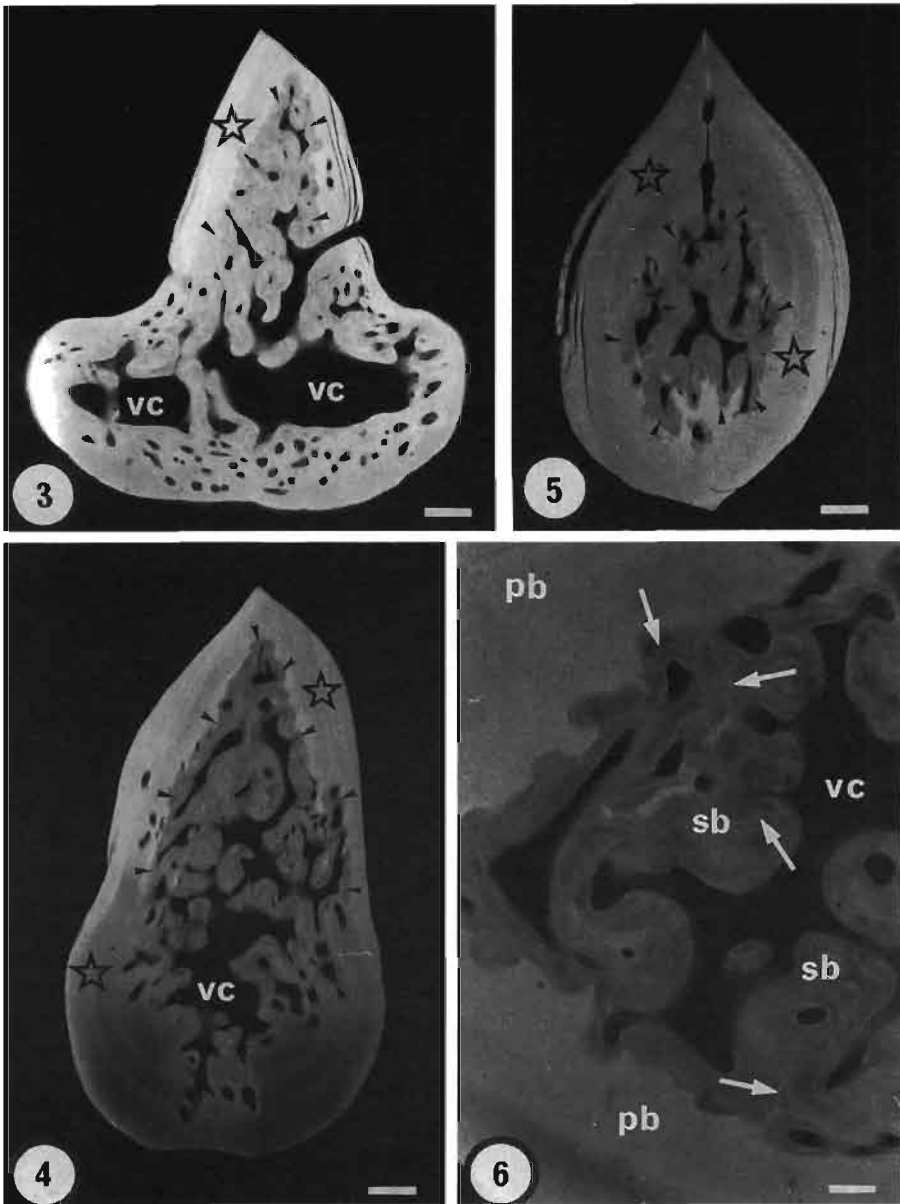
The aim of this work is to specify the structure of the caudal spine in *Ctenochaetus striatus* with the histological study of undemineralized material.

## MATERIAL AND METHODS

### Material

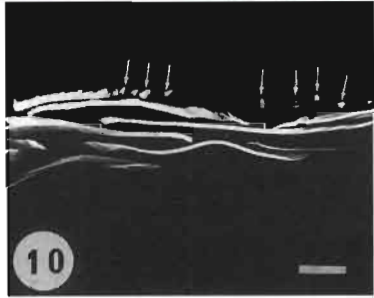
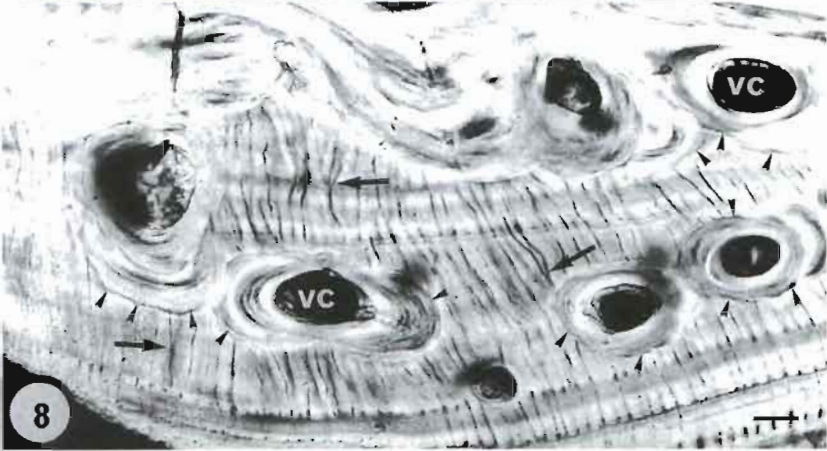
We have studied the histological structure of caudal spines in specimens of *Ctenochaetus striatus* donated to us by Dr. R. Galzin. Six specimens (192 to 236 mm TL; 117 to 170 g) caught in Moorea atoll (French Polynesia) were fixed in neutral formaline.

*Ctenochaetus* larvae were not available, but some larvae of *Acanthurus* sp. (I.19729.039) from the Australian Museum in Sydney have been studied with the aim of performing developmental studies. One larva (11 mm SL) of *Acanthurus* sp.



Figs 3, 4, 5. - *Ctenochaetus striatus* (microradiograph). Transversal ground section in proximal (Fig. 3), mid (Fig. 4) and distal (Fig. 5) levels (see Fig. 1, respectively a, b and c) of a caudal spine. The center of the spine is made of vascular secondary bone (arrowheads) whereas cortical bone is avascular primary bone (stars). (vc = vascular cavity). Bar = 100  $\mu$ m.

Fig. 6. - *Ctenochaetus striatus* (microradiograph). Detail of a transversal ground section showing avascular primary bone (pb) and the less mineralized secondary bone (sb). Arrows point to cementing lines. (vc = vascular cavity). Bar = 40  $\mu$ m.



(I.25355.10), and three larvae (9.2, 11 and 17.5 mm SL) of *Naso* sp. (I.24548.009) have been stained (alcian blue - alizarine red S) and the flesh has been cleared with glycerine, following Simons and Van Horn technics (1970/71).

### Methods

Caudal spines were dissected with the surrounding skin, dehydrated, then embedded in stratyl resin (Chronolite 2060). Sections were made with a sawing machine (Isomet). These sections about 200  $\mu$ m thick were microradiographed (SO 643 film) and grounded until they reached a thickness of 80  $\mu$ m. Observations were made with a light microscope in natural and polarized transmitted light.

One caudal spine was cleaned with 6% sodium hypochloride solution at room temperature for a few minutes, washed in distilled water, dehydrated in absolute ethanol, dried and coated with evaporated gold, and observed in a JEOL-SEM-35 apparatus.

## RESULTS

### Histological structure of the caudal spine

The morphology of caudal spines of surgeonfish is similar to that described by Monod (1959) in *Acanthurus monroviae*. In *Ctenochaetus striatus*, the caudal spines are constituted of acellular bone.

The body of the spine is divided in two parts: the first one, peripheral, is constituted of cortical, primary, avascular bone (Figs 3-5) and the second one, more central, the medulla, is made up of secondary vascularized bone (Fig. 6). The medullar bone is clearly less mineralized than the cortical bone and numerous cementing lines are seen. This means that the medulla is constituted of secondary bone that results from remodeling of primary bone (Fig. 7). The cortical bone shows concentric growth marks (Fig. 7) that indicate an appositional growth of the caudal spine. This bone is crossed by osteoblastic canaliculi (Fig. 8) some of them being branched. A SEM study of the surface of the caudal spine shows regularly distributed tiny holes (Fig. 9) which are 0.5 to 1.5  $\mu$ m in diameter. These holes are the apertures where the cytoplasmic prolongations of the osteoblasts penetrate primary bony tissues (see Meunier, 1983; Meunier and François, 1992).

Even without quantitative measurements (quantitative microradiography), we can assume that mineralization of primary bone is normal and probably similar to mineralization of the marine acellular bone.

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Fig. 7. - *Ctenochaetus striatus*. a) natural light, b) polarized light. Ground sections showing growth lines (arrows) in cortical bone and some secondary osteones (black and white arrowheads). Bar = 25  $\mu$ m.

Fig. 8. - *Ctenochaetus striatus*. Ground section, natural light. Detail showing ramified canaliculi (arrows) that house osteoblastic processes. Bony tissue of secondary osteons that are demarcated by cementing lines (arrowheads) is deprived of osteoblastic processes. Bar = 25  $\mu$ m.

Fig. 9. - *Ctenochaetus striatus*. MEB. Mid anterior region. Numerous holes (arrows) are seen at the surface of bone. Bar = 10  $\mu$ m.

Fig. 10. - *Ctenochaetus striatus*. Microradiograph. Caudal skin. Transversal ground section. Arrows point to cteni of scales. Bar = 100  $\mu$ m.

### Appearance of the caudal spines during ontogeny

On the stained *Acanthurus* larva (11 mm long) the caudal spine is already present. It is a tiny spine inserted on a round base. The surrounding scales are oval-shaped ctenoid scales and they show one "stout" ctenius on their posterior margin. The same observations have been made on the unstained shorter larva (5 mm SL). Thus, the differentiation of the caudal spine, as well normal scales, is already in progress at this stage. Similar results have been obtained on the three *Naso* larvae.

## DISCUSSION

Our histological observations show that the caudal spines in *Ctenochaetus striatus* are constituted of typical bony acellular bone, i.e., bone lacking true osteocytes (Kölliker, 1859; Stephan, 1900; Moss, 1961a, 1961b, 1963, 1965; Weiss and Watabe, 1979; Parenti, 1986; Meunier, 1987). These mineralized tissues show various structural indications characterizing a living bony tissue: growth marks, remodeling (Francillon-Vieillot *et al.*, 1990; Ricqlès *et al.*, 1991). However, primary bone tissue houses cytoplasmic processes but not secondary bone. The lack of cellular processes in secondary bone was already mentioned in the spiny rays of *Lethrinus nebulosus*, another perciform (Meunier, 1983). Therefore the caudal spine of the surgeonfish can be considered as a true bony structure.

To compare caudal spine structure with the various parts of an elasmoid scale we now present the main characteristics of scales of *Ctenochaetus striatus* (Meunier, in prep.) The structure of the scales shows the two typical layers of the elasmoid scales: an outer ornamented layer which is relatively homogeneous, overlaying a stratified basal plate. A limiting layer that frequently overlies the outer layer in various teleosts scales seems to be lacking, but this must be checked at the ultrastructural level. The outer layer is thick in the posterior area especially at the level of the ctenii (Fig. 10). The anterior area is ornamented by more or less concentric ridges. Scales of *C. striatus* are ctenoid, i.e., their posterior margin shows a row of spines caudally oriented. These spines are regularly replaced by new ones inserted between the older ones which lose their apical spine, probably because of an osteoclastic activity. They have the same structure as the ornamented layer and, in cross sections of ctenii, some scarce osteoblastic canaliculi are observed. The basal plate of the scales is constituted of isopedine (see Meunier, 1984), partly mineralized, but before the mineralizing front, Mandl's corpuscles are small or lacking.

In the adult fish the strict homology between a normal scale and a caudal spine is not visible. Moreover the caudal spine, as the scales, differentiates early during the ontogeny, probably after the *acronurus* stage, during the metamorphosis (Randall, 1961). In *Naso* the caudal spine would form from existing scales in opposition to *Acanthurus* where it would form directly without an unspecialized stage (Leis and Richards, 1984). If the caudal spine is a modified scale in *Ctenochaetus striatus*, which we do not dispute if we refer to the present study, the ontogenetical signals probably start rather early during ontogeny. The hypothesis we want to propose is that the caudal spine could be equivalent to the first ctenius that forms on the posterior margin of the young scales. It could differentiate into a specialized structure, the other parts of a scale remaining undifferentiated or disappearing quickly during the ontogeny of the caudal spine. Thus, it means that this problem should be solved by studying comparatively the scales and the caudal spine ontogeny in surgeonfish larvae, at least from the *acronurus* stage, especially with transmission electron microscopy.



**Acknowledgements.** - We thanks Prof. René Galzin for supplying the *Ctenochaetus* specimens and Dr. Jeffrey M. Leis for the loan of *Acanthurus* and *Naso* larvae. We are grateful to Dr. Alexandra Quilhac for improving the English manuscript.

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## **SOME MORPHOLOGICAL AND HISTOLOGICAL ASPECTS OF HYPEROSTOSIS IN THE EASTERN PACIFIC MARINE FISH *PRIONOTUS STEPHANOPHRYS* LOCKINGTON, 1880 (TRIGLIDAE)**

by

François J. MEUNIER (1, 2), Philippe BÉAREZ (1) & Hélène FRANCILLON-VIEILLOT (2)

**ABSTRACT.** - All the fish studied show the same hyperostotic bones: both frontals, the ethmoid complex, both operculars and one or two caudal vertebrae. Hyperostosis was always found in the 21st vertebra and sometimes in the 20th and 22nd. The cranial bones show a typical and regular thickening. On the contrary, in the case of the vertebrae, this thickening concerns each side of the centrum only. The swelling of all these bones is the result of an active osteogenesis associated with resorbing processes resulting in the deposition of spongy acellular bone. This cancellous bone is surrounded by a thin cortical primary bone. Thus, the histological characteristics of the swollen bones of *Prionotus stephanophrys* are the same as those already described in the majority of other hyperostotic species, regardless of the bones involved. The causality and the biological signification of the hyperostotic phenomenon in *P. stephanophrys* seem unclear in the current state of knowledge but, given the particularly specific vertebral swelling of this species, this fish could represent an interesting model to study the etiology of hyperostosis.

**RÉSUMÉ.** - Quelques caractères morphologiques et histologiques de l'hyperostose chez un poisson marin du Pacifique Est, *Prionotus stephanophrys* Lockington, 1880 (Triglidae).

Tous les spécimens étudiés montrent les mêmes hyperostoses: les deux frontaux, le complexe ethmoïdien, les deux operculaires et une ou deux vertèbres caudales. C'est la 21ème vertèbre qui est hyperostotée ainsi que la précédente ou la suivante dans certains cas. Les os crâniens présentent un épaississement caractéristique et pour les vertèbres c'est seulement le centrum qui est hyperostoté. L'épaississement de tous ces os est le résultat d'une ostéogenèse active associée à des processus d'érosion qui produisent de l'os spongieux acellulaire. Cet os spongieux est entouré d'une fine corticale d'os primaire. Les caractéristiques histologiques des hyperostoses de *Prionotus stephanophrys* sont donc les mêmes que celles de la majorité des autres espèces déjà étudiées quels que soient les os hyperostotés. Les causes et la signification biologique du phénomène hyperostotant chez *Prionotus stephanophrys* ne sont pas claires en l'état actuel des connaissances; mais compte tenu de son développement particulier sur les vertèbres caudales cette espèce apparaît comme un modèle intéressant pour une étude étiologique de l'hyperostose.

**Key-words.** - Triglidae, *Prionotus stephanophrys*, Bone, Hyperostosis.

The lumptail searobin (*Prionotus stephanophrys*) is a common triglid of the eastern Pacific from California to Peru and sometimes as far as Chile (Samamé *et al.*, 1983;

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Huidobro-Campos and Schmitter-Soto, 1993; Schmitter-Soto and Castro-Aguirre, 1996; Béarez, 1996a). This fish, which attains in average 35 cm, but was recorded 43 cm (TL) (Franke and Acero, 1996), lives on the continental plate between 20 and 150 m deep (Schmitter-Soto and Castro-Aguirre, 1996). About fifteen years ago, numerous dead specimens were found on the shore of the Peruvian coast, probably as a result of an El Niño phenomenon. One peculiarity of these fishes was the presence of hyperostotic vertebrae (C. de Muizon, pers. com.). Hyperostosis had already been noticed in triglids (Korschelt, 1940; Smith-Vaniz *et al.*, 1995) and they are also known in two species from the western Pacific: *Pterygotrigla hemisticta* and *P. ryukyuensis* (Richards, pers. com.); the latter species shows tremendous swellings of the head bones and first dorsal fin spines whereas the swellings are less severe in *P. hemisticta* and restricted to head bones (Richards, pers. com.). But none of these triglids have been studied in detail so far.

Recently, one of us collected several specimens on the Ecuadorian coast (Béarez, 1996b). All the specimens exhibited hyperostosis and this gave us the opportunity to study their morphological and histological characters and to compare them with other well known hyperostotic species: carangids, haemulids, sciaenids, sparids, etc. (e.g., Chabanaud, 1926; Desse *et al.*, 1981; Gaudie and Czochanska, 1990; Meunier and Desse, 1994; Smith-Vaniz *et al.*, 1995; *inter alia*). Such comparative studies may lead to a better understanding of the causality of this phenomenon in Teleostei as the etiology of hyperostotic phenomenon being unclear in fishes so far.

## MATERIEL AND METHODS

### Material

We have studied 11 specimens from 208 to 329 mm total length (weight: 92 to 360 g).

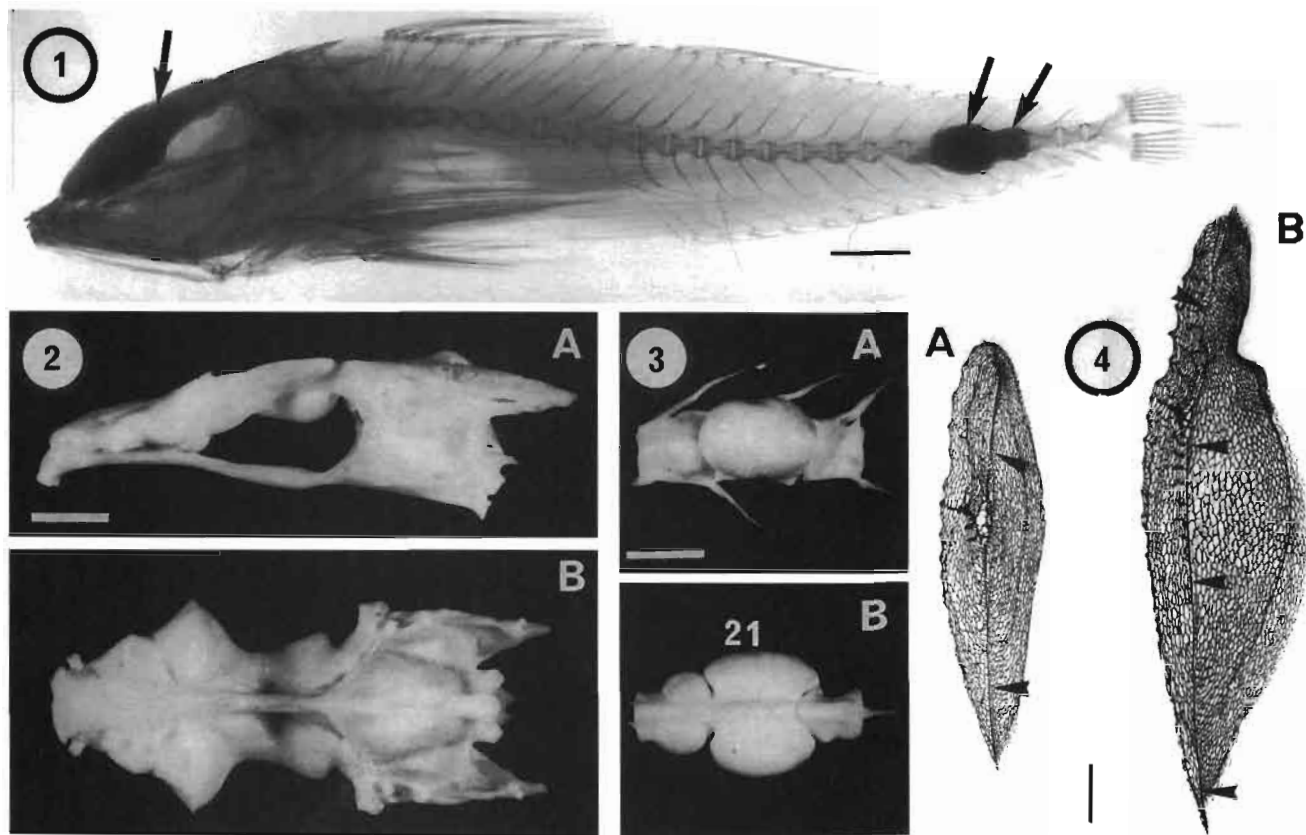
### Methods

Two specimens (MNHN 1997-4082) were radiographed in a Faxitron apparatus. The skeleton of the remaining fish were cleaned after boiling and then dried. Ethmoid bones, operculars and various vertebrae (the 21st, the partly hyperostotic 20th and non hyperostotic ones) were embedded in stratyl resin (Chronolite 2060). Sections were performed with a sawing machine (Isomet). These sections, about 200 µm thick, were microradio-graphed (SO 643 film) and then grounded until they reached a thickness of 80 µm. Observations were made with a light microscope in natural and polarized transmitted light.

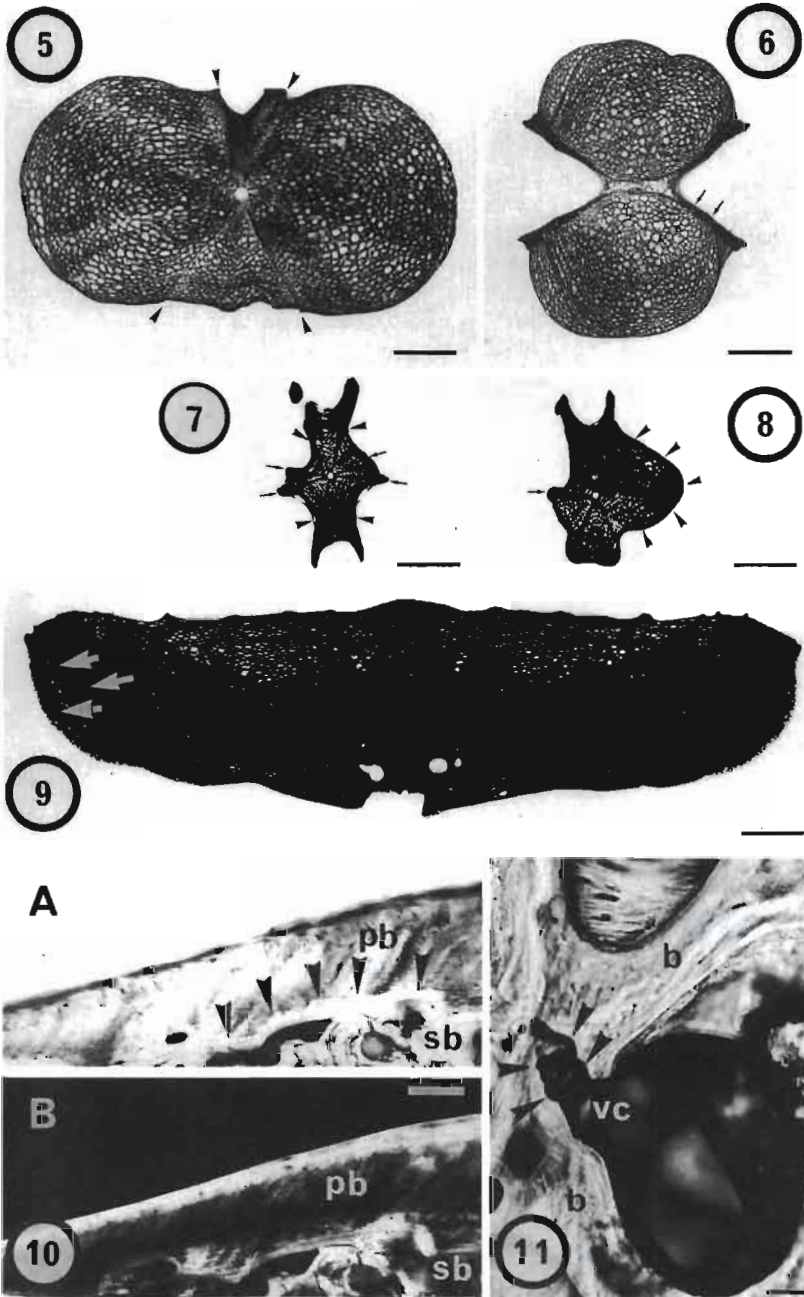
## RESULTS

### Morphology

All the 11 specimens exhibited the same hyperostotic bones on both radiographs (Fig. 1) and dry skeletons (Figs 2, 3): the two frontals, the ethmoid complex, the two operculars and one or two caudal vertebrae. The 21st vertebra always showed hyperostosis as a swelling of the centrum only, while the neural and hemal arches and spines were normal. In some cases the 22nd vertebra, sometimes the 20th, showed a tiny swelling of the half centrum nearest (Fig. 3). The cranial bones (frontal and ethmoid) show a typical thickening of their whole part but for the opercular bones the swelling affects essentially the internal (concave) face.



Figs 1-4. - *Prionotus stephanophrys*. Fig. 1. - "Negative" radiograph (mineralized parts in black); specimen MNHN 1997-4082. Arrows point to swollen bones. Bar = 1 cm. Fig. 2. - Neurocranium. A) Lateral view; B) ventral view. Bar = 1 cm. Fig. 3. - Vertebrae 20 to 22. A) lateral view; B) ventral view. Bar = 1 cm. Fig. 4. - Opercular. Ground sections ("Negative" microradiograph). A) Anterior level; B) mid level. In the center of the opercule the thin compact bony initium (arrowheads) on which spongy bone has been deposited, can be seen. Bar = 2 mm.



### Histology

In *P. stephanophrys*, the general structure of all these swollen bones looks strikingly similar, namely a great development of spongy bone (Figs 4-7). This spongy bone is surrounded by a rather thin layer of avascular bone that constitutes cortical bone. Nevertheless, cortical bone is lacking in hyperostotic operculars (Fig. 4). Hyperostotic as well as non-hyperostotic bone is acellular, in other words osteocytes are lacking in the bony tissue.

On sections of the hyperostotic vertebrae, the vascular bone that surrounds the chordal canal is crossed by bony radial arches, the bony tissue of which is avascular (Fig. 5). In fact, these arches constitute, with one or two pairs of horizontal (or septal) arches, the main part of a normal vertebra where they all sustain patches of vascular bone (Fig. 7). In hyperostotic vertebrae the radial arches have been embedded in a bulk of vascular bone; the septal arches have disappeared replaced by cancellous bone, probably because of remodeling (Fig. 8). The growth of these hyperostotic bony tissues is the result of the hyperactivity of osteoblasts.

The hyperostotic neurocranial bones (ethmoid complex and frontals) are particularly thick because of spongy acellular bone (Fig. 9).

The swelling of all these bones is the result of an active periostic osteogenesis that deposits primary pseudolamellar avascular bone. This cortical bone is progressively resorbed and it is replaced by cancellous bone (Fig. 10). In any case, a part of the cortical bone can be preserved from the resorption and this thin layer of avascular bone looks like growth marks (Figs 6, 9). The presence of Howship's lacunae on the walls of erosive bays indicates that the resorption is due to osteoclastic activity (Fig. 11). In the case of the operculars, primary bone is of the vascular type but local remodeling may also occur.

### DISCUSSION

As in numerous other hyperostotic teleostean fishes, *P. stephanophrys* develops hyperostoses on specific bones, the neurocranium and the 21st vertebra (see Desse *et al.*, 1981; Gauldie and Czochanska, 1990; Meunier and Desse, 1994; Smith-Vaniz *et al.*, 1995). The localization of the hyperostotic bones in the present species deserves some comment. Firstly, the neurocranium shows several swollen bones. It is generally the

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Figs. 5-11. - *Prionotus stephanophrys*.

Fig. 5. - Vertebra 21. Ground section ("Negative" microradiograph). Cross section. Arrowheads point to the radial arches of the native vertebra; compare with figure 7. Bar = 2 mm. Fig. 6. - Vertebra 21. Ground section ("Negative" microradiograph). Longitudinal section. The centrum is filled with spongy bone. The arrows indicate the region shown in figure 10. Bar = 2 mm. Fig. 7. - Non-hyperostotic caudal vertebra. Ground section ("Negative" microradiograph). Cross section. The arrowheads and the arrows point respectively to the radial arches and to the horizontal arches of the centrum. Bar = 2 mm. Fig. 8. - Partly hyperostotic caudal vertebra. Ground section ("Negative" microradiograph). Cross section. Hyperostosis has developed on the right side (arrowheads) of the centrum, the horizontal arch of which has disappeared. On the contrary, on the left side, the horizontal arch is always present (arrow). Bar = 2 mm. Fig. 9. - Hyperostotic ethmoid complex. Ground section. Spongy bone is widely prevalent but some deep avascular bone layers are seen (arrows). Bar = 2 mm. Fig. 10. - Vertebra 21. Longitudinal ground section. A) transmitted natural light; B) polarized light. Detail showing remodeling of vertebral bone. Arrowheads point to the cementing lines between primary (pb) and secondary (sb) bone. Bar = 50  $\mu$ m. Fig. 11. - Vertebra 21. Longitudinal ground section (transmitted natural light). Arrows point to Howship's lacunae (b. = bone; vc. = vascular cavity). Bar = 25  $\mu$ m.

occipital crest of the cranium which is affected, the other bones being unmodified: Carangidae (Desse *et al.*, 1981; Smith-Vaniz *et al.*, 1995; see also Driesch, 1994). However other species develop spectacular hyperostoses in cranial bones, for example in various Haemulidae (for example *Pomadasys hasta* of the Indian Ocean), the large neurocranium of which is so strong that it is found in archeological sites (Meunier and Desse, 1994). Secondly, it is noteworthy that the strict localization of hyperostosis is on the same caudal vertebra, i.e., the 21st, with a possible extension to the two nearest ones (20th and 22nd). The questions are: why do some bones constantly develop hyperostosis and some do not in a given species? Why is this development species-specific? Our studies confirm the taxonomic aspect of the hyperostotic phenomenon because it always affects the same bones in a given species (Driesch, 1994) but the phenomenon differs from one species to another. This suggests that hyperostosis seems to have a genetic origin.

The histological characteristics of the swollen bones of *P. stephanophrys* are similar to those already described in other hyperostotic extant species, and are independent of the anatomical localization of the hyperostotic bones. The spectacular swelling of the bones results from an activation of osteogenesis that yields primary spongy bone directly, or after an associated remodeling (Fierstine, 1968; Desse *et al.*, 1981; Gauldie and Czochanska, 1990; Meunier and Desse, 1994; Smith-Vaniz *et al.*, 1995). Hyperostotic bones show mostly a spongy structure (Kaiser, 1960; Fierstine, 1968; Desse *et al.*, 1981; Gauldie and Czochanska, 1990), except in the fossil cyprinodontid *Aphanius crassicaudus*, which has swollen avascular bones (Meunier and Gaudant, 1987), but a small length not more than 5 to 6 cm (TL).

Dealing with the question of the causality and the biological significance of the hyperostotic phenomenon in *P. stephanophrys* as in other fish, there are no clear and satisfactory explanations yet for any hyperostotic case recorded so far, but we can discuss some hypotheses thanks to our current knowledge. Various hypotheses have been indeed proposed to explain the development of these bones: aid in fin erection, aid in neutral buoyancy, ageing action on bone, reaction to high temperatures, metabolic abnormality, pathogenic phenomenon, genetic factors, etc. (see review in Meunier and Desse, 1986).

Do hyperostotic bones correspond to pathological features? Except for Bhatt and Murti (1960) the authors agree that hyperostosis are not pathological formations (Olsen, 1971; Desse *et al.*, 1981; Gauldie and Czochanska, 1990). It also appears that fishes with such swollen bones show a normal behaviour (Johnson, 1973). For example, hyperostotic processes seem inescapable in the jack mackerel, *Trachurus trachurus* (Carangidae), since a high number of individuals show swollen bones at the end of their life (Desse *et al.*, 1981). As these fishes do not show abnormal behaviour, we can consider that the phenomenon is not pathologic, at least in this species and, possibly, in the whole carangid family. We think that it is the same in *P. stephanophrys*. In a more general way, the etiology of the hyperostotic phenomenon in fishes is poorly known. Presently, this phenomenon occurs mainly among marine species and in the majority, among species with acellular bone (Desse *et al.*, 1981; Meunier and Desse, 1986). However, hyperostotic bones may have been recorded in some less highly evolved species: for example the marine Pacific catfish: *Bagre pinnimaculatus*, *B. panamaensis* (Ariidae) and *Cynoponticus coniceps* (Muraenesocidae) (Meunier and Béarez, unpubl. data). These three species have cellular bone. Hyperostosis was also described in a fossil Clupeidae of the Miocene (Gaudant and Meunier, 1996), the bony tissue of which shows osteocytes.



The hyperostotic bones increase in size (possibly in number for some species) along with the growth of the fish (Desse *et al.*, 1981; Meunier and Gaudant, 1987; Gauldie and Czochanska, 1990), but the correlation between these factors is low (Gauldie and Czochanska, 1990). The more intensive analytical studies have been made on *Trachurus trachurus*, *T. mediterraneus* (Desse *et al.*, 1981; Laroche *et al.*, 1982) and *Chrysophrys auratus* (Gauldie and Czochanska, 1990). These studies have failed to show significant differences between normal and hyperostotic bones. For example, there was no difference in fatty acid composition between the hyperostotic and normal vertebrae in *Chrysophrys auratus* (Gauldie and Czochanska, 1990) whereas Breder (1952) had suspected that fat in bones may play an important function. In the same way, there was neither significant variations of the calcemy, of the phosphatemy and of the calcitonin content, nor of the physical characteristics of the bony mineral between hyperostotic and normal *Trachurus trachurus* (Desse *et al.*, 1981; Laroche *et al.*, 1982).

In the case of a hydrodynamic hypothesis an unresolved question is the fact that the hyperostotic phenomenon affects fish that show strikingly different morphologies: for example the carangids *Caranx*, *Trachurus*, the triglid *Prionotus*, and the anguilliform muraenesocid *Cynoponticus* are morphologically different and it can be assumed that their swimming processes are also quite distinct (Lindsey, 1978; Webb, 1978; Ramzu, 1994).

For the species where the hyperostotic process seems to be a "normal" condition, as in numerous carangids, or as in *Prionotus stephanophrys*, a hydromechanical function during ageing is favoured (Desse *et al.*, 1981). To test this hypothesis we need quantitative studies on the modification of the fish shape during their life. There might be close correlations between genetic factors (mainly highly derived teleost species with acellular bone are affected), physiological factors (mechanical and physiological constraints of bone tissues) and external factors (physico-chemical parameters of sea water). We also think that bones in fish that develop hyperostosis may give an unvarying structural response of bony tissues to variable causal constraints.

## CONCLUSION

Whereas the morphology and the histological structure of hyperostosis look very similar to those of other species studied in that regard, the causality and the biological significances of the hyperostotic phenomenon in *P. stephanophrys*, as in other hyperostotic fishes, seem unclear. However, the specific localization of vertebral hyperostosis in *P. stephanophrys* and the availability of this species suggest it offers a suitable model to study the etiology of this phenomenon.

**Acknowledgements.** - We are very grateful to Monique Margout for her efficient bibliographic help, to Alexandra Quilhac for improving the English manuscript and to Prof. A. de Ricqlès for scientific discussion.

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## MORPHOLOGICAL PARTICULARITIES OF THE HEAD IN FOUR CARAPIDAE (OPHIDIIFORMES)

by

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**ABSTRACT.** - A study of the skull and the musculature of the oral and pharyngeal region of four adult Carapidae species (*Encheliophis boraborensis*, *E. homei*, *E. gracilis* and *Carapus acus*) has been undertaken to compare it with the diet related characters. The cephalic organization of *E. boraborensis* and *E. gracilis* seems related to diet (mainly fishes and shrimps for the first one and holothurian tissues for the other); these species are respectively commensal and parasitic. Although the feeding characters of *E. homei* and *C. acus* are closely similar to those of *E. boraborensis*, there are sparse observations of holothurian tissues in their stomach contents. It is suggested that these fishes are commensal when they are adults and have parasitic tendency when they are juveniles.

**RÉSUMÉ.** - Particularités morphologiques de la tête chez quatre Carapidae (Ophidiiformes).

Une étude du squelette et de la musculature des régions orale et pharyngienne de quatre espèces adultes de Carapidae (*Encheliophis boraborensis*, *E. homei*, *E. gracilis* and *Carapus acus*) a été réalisée afin de les comparer avec le régime alimentaire. L'organisation céphalique de *E. boraborensis* et *E. gracilis* semble être le reflet de leur alimentation (composée principalement de crustacés et de poissons pour le premier et de tissus d'holothuries pour le second): ils sont respectivement commensal et parasite. Bien que les caractères en relation avec la prise de nourriture d'*E. homei* et *C. acus* soient très semblables à ceux de *E. boraborensis*, quelques données ponctuelles rapportent la présence de tissus d'holothuries dans leurs contenus stomacaux. Ces poissons seraient des commensaux à l'état adulte et auraient des tendances parasitaires lorsqu'ils sont juvéniles.

**Key-words.** - Carapidae, *Carapus acus*, *Encheliophis boraborensis*, *Encheliophis homei*, *Encheliophis gracilis*, Cephalic morphology, Diet, Commensalism, Parasitism.

Most species of the Carapidae family have commensal or parasitic relationships either with an invertebrate echinoderm (Holothuroid or Asteroid) and/or a mollusc host (Markle and Olney, 1990). Stomach contents examination reveals their carnivorous life-style (Smith, 1964; Hipeau-Jacquotte, 1967; Trott, 1970; Dawson, 1971; Trott and Trott, 1972; Seymour, 1974; Gustato, 1976; Meyer-Rochow, 1977; Gustato *et al.*, 1979; Van Den Spiegel and Jangoux, 1989). Using a morphological approach, our aim is to contribute to the comprehension of carapids / holothurian host relationship. By studying the skeleton and the musculature of the oral and pharyngeal jaws in four carapids (*Encheliophis boraborensis*, *E. homei*, *E. gracilis* and *Carapus acus*), it is possible to

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confirm the diet and if a species is commensal or parasitic. Our choice was based on the availability of these four species and on their essentially similar behaviour towards their host. Moreover the three *Encheliophis* species are found inside the same holothurian species (*Bohadschia argus*). *E. boraborensis* is believed to eat mainly crustaceans and fishes (Trott, 1970; Van Den Spiegel and Jangoux, 1989), *E. homei* and *C. acus*, are thought to additionally consume the gonads, viscera, and respiratory trees of their host (Arnold, 1953; Smith, 1964; Hipeau-Jacquotte, 1967; Trott, 1970; Trott and Trott, 1972; Van Den Spiegel and Jangoux, 1989), and *E. gracilis* to feed solely on parts of the host (Strasburg, 1961; Smith, 1964; Branch, 1969; Trott, 1970; Van Den Spiegel and Jangoux, 1989).

## MATERIALS AND METHODS

Twelve *Encheliophis boraborensis* (TL: 13 to 30 cm), 13 *E. homei* (TL: 8 to 17 cm), and 5 *E. gracilis* (TL: 16 to 24 cm) adult specimens were collected in Hansa bay (Bismarck's sea, North of Papua New Guinea). They were found inside specimens of *Bohadschia argus* (a holothurian). The 8 specimens of *Carapus acus* (TL: 7 to 15 cm) were found in *Holothuria forskali* from the Mediterranean sea (S.T.A.R.E.S.O. station, Calvi, Corsica).

The carapids were preserved in 5% formaline or frozen at -20°C. Three individuals of each species were stained with Alizarin and Alcian blue according to Taylor and Van Dijk (1985) to reveal the skeletal structures. All fish were dissected and examined with a Wild M10 binocular coupled with a camera lucida.

### Abbreviations

A : adductor mandibulae A	A <sub>1</sub> : adductor mandibulae A <sub>1</sub>	A <sub>1</sub> : adductor mandibulae A <sub>1</sub>
A <sub>2</sub> : adductor mandibulae A <sub>2</sub>	A <sub>2</sub> : adductor mandibulae A <sub>2</sub>	A <sub>3</sub> : adductor mandibulae A <sub>3</sub>
A <sub>3</sub> : adductor mandibulae A <sub>3</sub>	AA : articuloangular	art.pro.pmx.: articular process of the maxillary
asc.pro.pmx : ascending process of the premaxillary	BBR 1-4 : basibranchial 1 to 4	BH : basihyal
CBR 1-5 : ceratobranchial 1 to 5	DE : dentary	EBR 1-5 : epibranchial 1 to 4
HBR 1-3 : hypobranchial 1 to 3	HM : hyomandibular	HM pos. : Hyomandibular position
INEL : interarcual element	IO : interoperculum	lat.pro.pmx : lateral process of the premaxillary
LEAP : levator arcus palatini	LETH : lateral ethmoid	LEXT 1-4 : levator externus 1 to 4
Li. : ligament	LINT 2 : levator internus 2	LINT 4 : levator internus 4
MESO : mesopterygoid	META : metapterygoid	MX : maxillary
NCR : neurocranium	O : operculum	PA : palatine
PBR 1 : pharyngobranchial 1	PBR 2-4 : pharyngobranchial 2 to 4	PMX : premaxillary
PO : preoperculum	pa. pro : palatine process	Q : quadrate
R : rib	RC : rostral cartilage	RD : retractor dorsalis
SB : swim bladder	SCAP : pectoral girdle	SO : suboperculum
SOP : primary sound-producing muscle	SYMP : symplectic	I, II, III, IV : vertebra

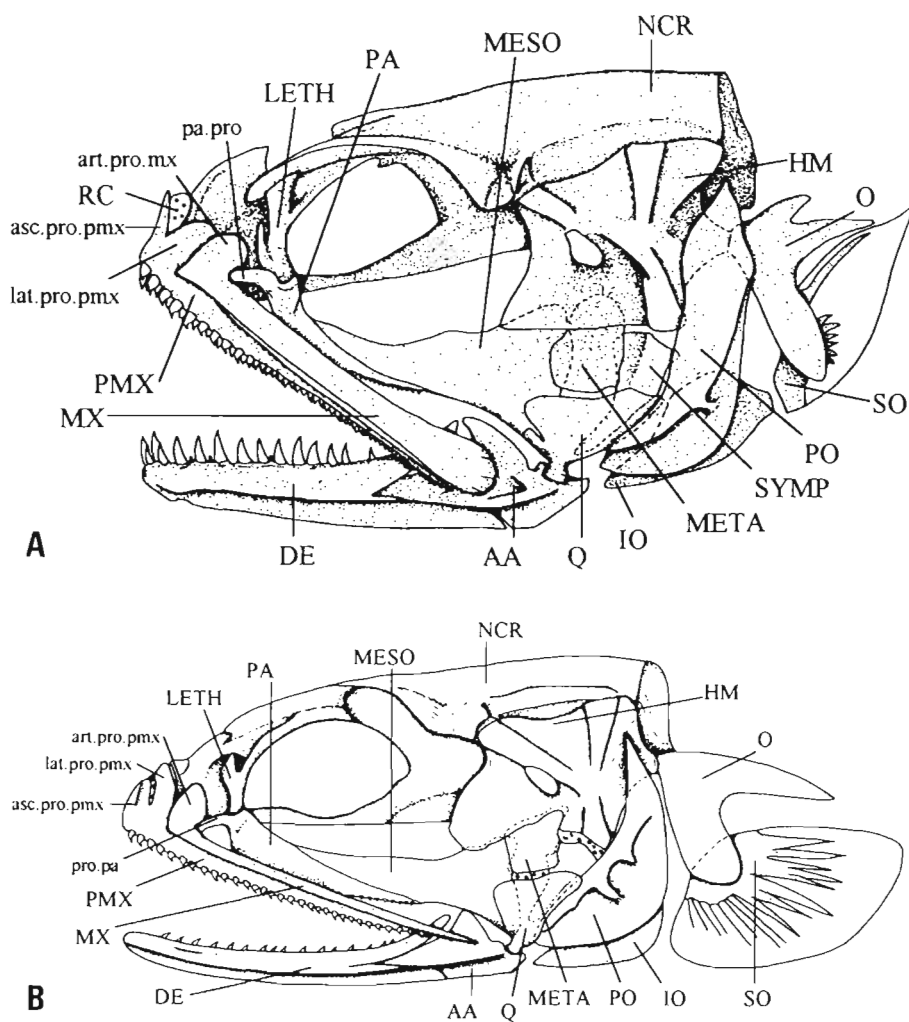


Fig. 1. - Lateral view of the cephalic skull in *Encheliophis homei* (A) and *E. gracilis* (B).

## RESULTS

### Skull of the oral region

In all four species, the premaxillary bears a row of external cardiform teeth (Fig. 1) and an additional two or three rows of conical internal teeth in *Encheliophis boraborensis*, *E. homei* and *Carapus acus*. The premaxillary of *E. gracilis* is also distinctive from the three other species by its close ascending and lateral processes (Fig. 1B). The articular process of the maxillary articulates on the anterior process of the palatine and caps the premaxillary. The ethmoid region of the four species has two ligaments (Li. 1 and 2) in common (Fig. 2). Li. 1 divides into two branches: the first branch attaches on the mesethmoid and on the base of the maxillary articular process and the sec-

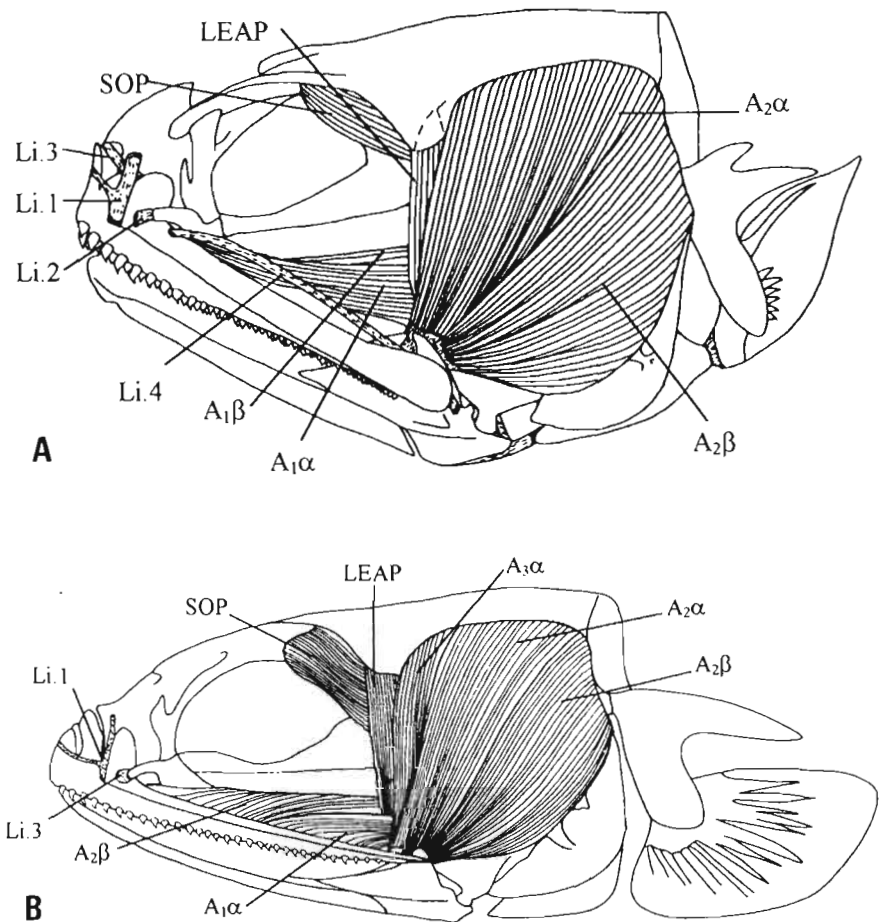


Fig. 2. - Lateral view of the *adductor mandibulae* muscles and of the *levator arcus palatini* muscle in *Encheliophis homei* (A) and *E. gracilis* (B).

ond branch originates on the first branch and runs to the opposite premaxillary ascending process. Li. 2 joins the anterior process of the palatine and the upper part of the maxillary. In addition there are two other common ligaments in *E. boraborensis*, *E. homei* and *C. acus*: the first one (Li. 3) attaches on the maxillary articular process and on the rostral cartilage; the second one (Li. 4) is fixed below the Li. 3 and attaches to the articulo-angular (Fig. 2A). The latter two are missing in *E. gracilis*. On the other hand, *E. gracilis* possesses short connective fibres (Li. 5) spread along the maxillary and premaxillary length that do not permit a separation between the upper jaw elements.

The dentaries are robust and toothed. *Encheliophis boraborensis*, *E. homei* (Fig. 1A) and *Carapus acus* possess a row of large external, incurved conical teeth and several smaller internal teeth; *E. gracilis* possesses only external teeth (Fig. 1B). Proportionately, the lower jaws of the latter are slender (less thick and the coronoid processes less developed).



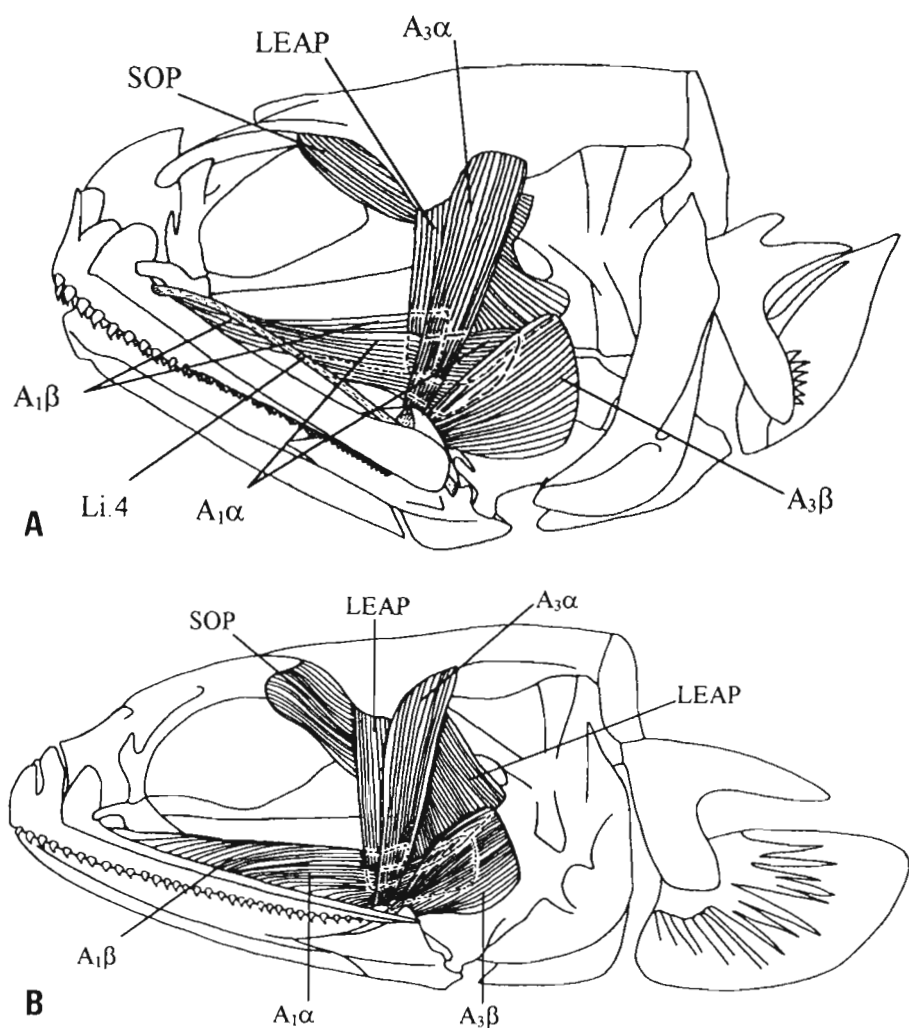


Fig. 3. - Lateral view of the *adductor mandibulae* muscles and of the *levator arcus palatini* in *Enchelio- phis homei* (A) and *E. gracilis* (B) when  $A_2$  bundles are removed.

In all four species the major characteristic of the suspensorium is in the large and reinforced hyomandibular. It articulates with the neurocranium along the otic region and displays two articular condyles: one on the sphenotic and the second on the pterotic. Both condyles are extended by thickenings that converge toward a third extending toward the symplectic. The quadrate shows a thickening in the continuation of the symplectic.

#### Musculature of the oral region

The *adductor mandibulae* muscle of the examined species has six bundles. Adductor  $A_2$  is the most external and is separated in two bundles:  $A_2\alpha$  and  $A_2\beta$  (Fig. 2). These muscles extend from the inner side of the dentary coronoid process to the pterotic and hyomandibular respectively. Adductor  $A_1$  lies under  $A_2$  and is divided in two bundles

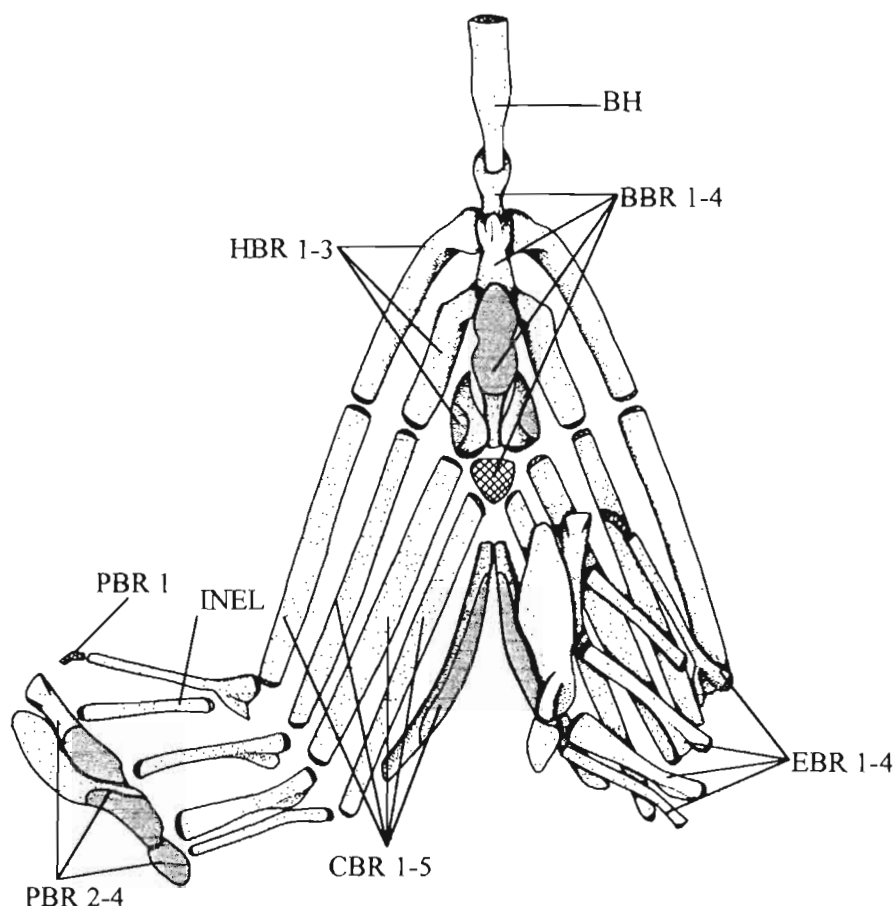


Fig. 4. - Dorsal view of the branchial basket in *Encheliophis boraborensis*. Grey surfaces represent the teeth positions and hatched surfaces the cartilage.

(Figs 2, 3). The first one ( $A_3\alpha$ ) inserts dorsally on the sphenotic and is ventrally in contact with  $A\omega$  adductor and the second one ( $A_3\beta$ ) inserts on the central part on the suspensorium (hyomandibular, metapterygoid and symplectic) and on A.

A new major difference appears with the disposition of the  $A_1$  bundles ( $A_1\alpha$  and  $A_1\beta$ ) between *E. gracilis* and the three other species (Figs 2, 3). In *E. boraborensis*, *E. homei* and *C. acus*,  $A_1\alpha$  inserts on the metapterygoid posteriorly and on ligament 4 (Li. 4) anteriorly.  $A_1\beta$  extends from the mesopterygoid to the inner face of the maxillary. In *E. gracilis*, insertions on the suspensorium are similar but none anterior, the two bundles insert along most of the length of the maxillary.

#### Functional features of the oral region

In *Encheliophis boraborensis*, *E. homei* and *Carapus acus*, the mouth opening shows a forward projection of the upper part of the premaxillary when the dentary is lowered. In addition to this movement, the posterior parts of the upper jaws move laterally. In

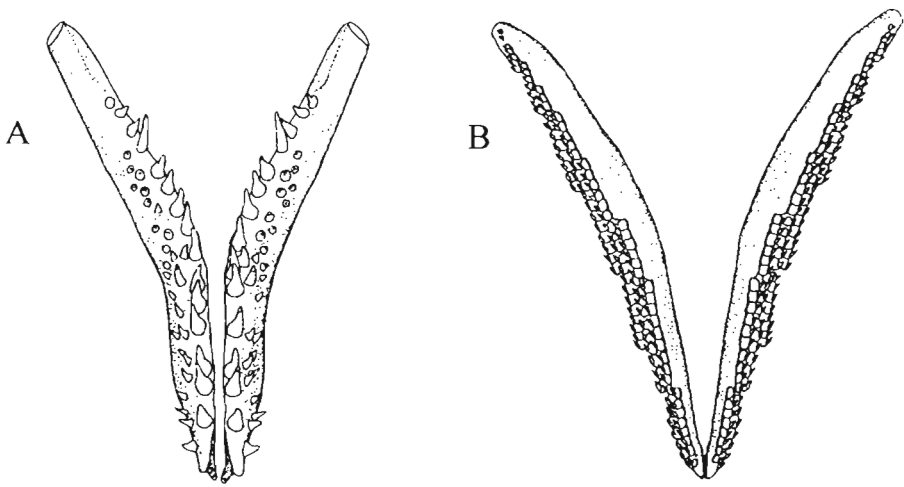


Fig. 5. - Dorsal view of the 5th pharyngobranchial in *Encheliophis boraborensis* (A) and *E. gracilis* (B).

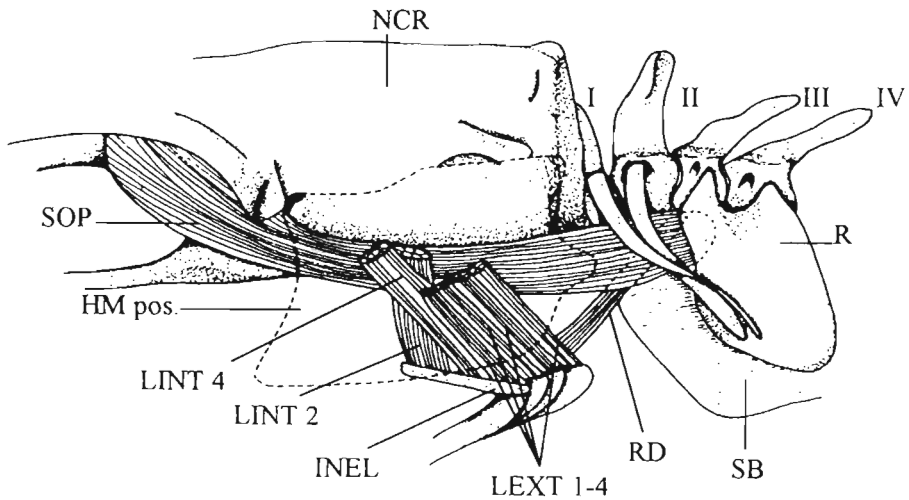


Fig. 6. - Lateral view of the upper pharyngeal muscles and of the sound-producing muscles in *E. homei*.

*E. gracilis*, there is no projection of the premaxillaries upon mouth opening and the mouth opens less widely. Furthermore, the premaxillaries and maxillaries do not show interdependent movements.

#### Skull of the pharyngeal region (Fig. 4)

The disposition and the form of the fifth ceratobranchials (= lower pharyngeal jaws, LPJ) differ between the four species examined. Generally speaking, in *Encheliophis boraborensis*, *E. homei* and *Carapus acus*, the 5th ceratobranchials are robust, their upper surface has big teeth on the inner side and small teeth on the outer side (Fig. 5A). These teeth are conical and are directed toward the back of the buccopharyngeal cavity. In *E.*

*gracilis*, the 5th ceratobranchials are flattened and separated from each other posteriorly. From a dorsal view, they slope laterally and have only cardiform teeth on their inner dorsal surfaces (Fig. 5B). In these four species, a membrane joins the right and left ceratobranchials.

In all four species, the 2nd, 3rd and 4th pharyngobranchials form the upper pharyngeal jaws (UPJ). *Carapus acus* has the largest 2nd pharyngobranchials. This element is smaller in *E. boraborensis* and *E. homei*, and *E. gracilis* has greatly reduced 2nd pharyngobranchials. In *E. gracilis*, the 4th pharyngobranchials are larger compared to the other species. Teeth are more numerous and more tapered in *E. gracilis*.

In all examined species, there are two characteristics (Fig. 4): (A) the 1st epibranchial does not connect with the 2nd pharyngobranchial. This role is assumed by an interarcual element (Allis, 1915) articulated on the one hand on the 1st epibranchial and on the other hand on the 2nd pharyngobranchial; (B) the 1st pharyngobranchial does not connect the neurocranium but is reduced and lost in conjunctive tissue.

Note: *E. boraborensis* and *C. acus* also have teeth on the 3rd basibranchial and on the 3rd hypobranchials, whereas *E. homei* only has teeth on the 3rd basibranchial. These teeth are missing in *E. gracilis*.

#### Musculature of the pharyngeal region (Fig. 6)

The ventral musculature of the branchial basket is that usually encountered in the Teleostei and is not described here. However, it is important to note that (A) the *levator interni* and *externi* originate on the inner side of the hyomandibular (except some fibres of the 3rd *levator internus* that attach to the neurocranium); (B) there is no *levator posterior* and (C) the *retractores dorsalis* muscles run obliquely between the primary sound-producing muscles and are attached posteriorly to the lower surface of the 2nd and 3rd vertebrae.

### DISCUSSION

Several characters are indicative of a carnivorous lifestyle in all four species. (1) There are major sets of conical teeth on the vomer (Arnold, 1953; Trott, 1970; Williams, 1984) palatines and jaws (Fig. 1); (2) There is a wide-split mouth with robust dentaries; and (3)  $A_2$  and  $A_1$  form a very powerful set of muscles inserting on the suspensorium, as in most teleosts (Liem, 1970; Lauder and Liem, 1981; Vandewalle *et al.*, 1995), but also with fibres on the neurocranium (Fig. 2). Vandewalle *et al.* (1982) suppose that such insertions are indicative of a prey catching by grasping. In addition, the powerful *adductores mandibulae* are associated with hyomandibular thickenings that are believed to be necessary to prevent bone deformation (Osse, 1969; Dutta, 1975; Vandewalle, 1978). The bisector of the angle formed by thickenings 1 and 3 points toward the coronoid processes of the dentary (short and near the quadrate); its position varies little during mouth opening (Fig. 7). The direction of this bisector is thus probably that of the force exerted on the hyomandibular and mandible by contraction of  $A_2$  and  $A_3$ . If so, the 1 and 3 thickenings might a response to the stress imposed by these muscles. The hypothesis that the force exerted by  $A_2$  and  $A_3$  is in the direction of the above-mentioned bisector is supported by the fact that the latter is also practically parallel to the quadrate thickening. It seems more difficult to explain thickening 2. Its presence probably reflects the added effects not only of  $A_2$  and  $A_3$  muscle contraction and the contraction of *levator arcus palatini*, and *adductor palatini*.

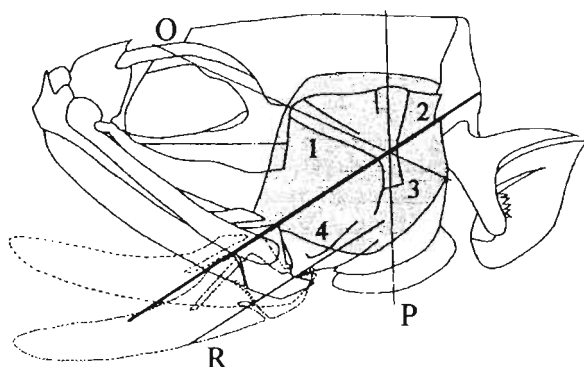


Fig. 7. - Simplified lateral view of the head in *Carapus acus*. Numbers 1 to 4 indicate the suspensorium thickenings; the grey surface represents the position of the  $A_2$ - $A_3$  muscles. The bold line is the bisector of the angle formed by O and P; R is the parallel to the bisector running through the quadrate-dentary joint.

Among these four species, anatomical characters seem related to their way of life. Carnivorous features are more pronounced in *Encheliophis boraborensis*, whose musculature, jaws, and suspensorium are the best developed, allowing the capture of "tough" prey, such as fishes and crustaceans. This is in keeping with stomach contents analyses of this species. Although these characters are somewhat less developed in *Carapus acus* and *E. homei*, they should be able to feed on prey with an endo-and/or exoskeleton. On the other hand, *E. gracilis* has thin buccal parts, uniserial set of teeth on the jaws and palatines, and maxillary ending in a spine (Fig. 1B). This morphology suits the diet which consists of soft food (holothurian tissues). These diet differences among species also relate to the mouth opening. The fact that *E. boraborensis*, *E. homei* and *C. acus* can protrude their upper jaw allows them to have a wide mouth opening. This protrusion is possible because the buccal parts are quite independent (Schaeffer and Rosen, 1961; Alexander, 1967; Lauder and Liem, 1981). On the other hand, *E. gracilis* displays no protrusion and no wide opening mouth. The most important factor of this feature seems to be the lack of mobility between maxillaries and premaxillaries: they are joined by dense connective tissues (Li. 5) along their entire length. In addition, the upper jaws do not have much mobility because the  $A_1$  bundles are inserted along the entire maxillary length and not on a ligament (Li. 4) and because they are concealed and confined by skin.

A wide mouth opening with a small protrusion is found in the three species that eat crustaceans and fishes. It permits the capture and retention of larger prey. On the other hand, a wide mouth opening is not necessary in the case of *E. gracilis*: in the host (and victim), there is no probability to lose the gonad or the viscera of the holothuroid. In addition the more numerous cardiform teeth of *E. gracilis* could consist in a sawing system that cuts soft tissue.

The four species possess two sound-producing muscles (Courtenay and McKittrick, 1970) that originate on the orbital roof, run ventrally alongside the neurocranium and attach to the swim bladder (Fig. 6). The topographic situation of the muscles could have two consequences at the branchial basket level. (1) It could indicate that the 1st pharyngobranchials are not involved in the suspension of the upper pharyngeal elements which do not have rigid suspension; (2) the remarkable origin sites of the *levatores* on the inner side of the hyomandibular and not on the neurocranium as is the case in all Teleosteans

known from this point of view (Holstvoogd, 1965; Nelson, 1967; Lauder, 1983; Liem and Sanderson, 1986; Claes and De Vree, 1992; Vandewalle *et al.*, 1995) is also probably linked to the presence of the sound-producing muscles. Their insertion on a mobile element could have functional implications. *Encheliophis boraborensis*, *E. homei* and *C. acus* all have the upper jaw teeth directed toward the rear and the inside, the strength of 5th ceratobranchials which have close-set sharp teeth, the thinness of the 4th *levator interni*, the presence of the *retractores dorsalis* on the one hand and the possible presence of entire prey in the stomach contents on the other hand suggest that the pharyngeal jaws are only able to transport food. In *E. gracilis*, weaker and more widely spread lower pharyngeal jaws with cardiform teeth as opposed to the longer teeth of the upper pharyngeal jaws could be related to cutting soft prey in addition to the transport movements.

All the *E. gracilis* stomach contents showed the exclusive presence of holothurian tissue as already reported in the literature (Strasburg, 1961; Smith, 1964; Branch, 1969; Trott, 1970; Van Den Spiegel and Jangoux, 1989). This is in accordance with our cephalic morphological study that shows poorly developed and somewhat different oral and pharyngeal carnivorous features in comparison with the three other species.

*Encheliophis boraborensis* has the most highly developed carnivorous characters and feeds only on non-holothurian tissues caught by grasping (Trott, 1970; Van Den Spiegel and Jangoux, 1989). Most authors have established that *C. acus* and *E. homei* feed on crustaceans or fishes (including cannibalism) (Arnold, 1956; Smith, 1964; Hipeau-Jacquotte, 1967; Trott, 1970; Trott and Trott, 1972; Van Den Spiegel and Jangoux, 1989). However, Arnold (1953) and Hipeau-Jacquotte (1967) mention the presence of holothurian tissues in, respectively, *C. acus* and *E. homei*, but these authors have combined data from juvenile and adult exemplars. It seems that young individuals feed on holothurian tissues then modification of the diet occurs in the adult stage when non-holothurian tissues are the principal source of food. Our cephalic examinations support this hypothesis because these fishes have morphological features closely similar to those of *E. boraborensis*: set of teeth on the dentaries, protrusion ability, muscular organization, etc. It would be interesting to know the diet of young *E. boraborensis* in order to see if they feed on holothurian tissues. If so, *E. boraborensis*, *E. homei* and *C. acus* have three ways of life during their life. They are free living and planktivorous (Williams, pers. com.) during their pelagic larval stage; they enter Holothuroid and are parasitic during their juvenile stage; they continue to enter Holothuroid during their adult stage but the host is used as a shelter. *Encheliophis gracilis* has lost this third way of life and is adapted to the parasitism.

**Acknowledgements.** - The authors thank C. Michel and S. Houbart (Aquarium, Liège, Belgium), J.M. Ouin and G. Seghers (Laing Island Biological Station, Papua New Guinea), and Dr. D. Bay (S.T.A.R.E.S.O., Calvi, France) for helping to fish the Carapidae specimens and Mrs K. Das for the linguistic assistance. This work is supported by grant n°2.4560.96. from the Belgian "Fonds National de la Recherche Scientifique".

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### **Symposium 3**

## **PELAGIC FISHES**

Chairperson: **Alexander HULLEY**

- COLLETTE B.B. - Mackerels, molecules, and morphology. 149-164  
*Maquereaux, molécules et morphologie.*
- MCDOWALL R.M. & A.L. STEWART. - Further specimens of *Agrostichthys parkeri* (Teleostei: Regalecidae), with natural history notes. 165-174  
*Notes d'histoire naturelle sur quelques spécimens d'Agrostichthys parkeri (Teleostei: Regalecidae).*



## MACKERELS, MOLECULES, AND MORPHOLOGY

by

Bruce B. COLLETTE (1)

**ABSTRACT.** - Current classification of the Scombridae, based largely on morphology, is compared with recent molecular studies in nine cases. I. The wahoo, *Acanthocybium solandri*, is confirmed as a scombrid, not a billfish. II. Relationships within the Thunnini are confirmed with *Auxis* the sister-group of the rest of the tribe, and then *Euthynnus*, *Katsuwonus*, and finally, *Thunnus*. III. Monophyly of the genus *Thunnus* is confirmed. IV. Monophyly of the subgenus *Neothunnus* of the genus *Thunnus* is confirmed but monophyly of the subgenus *Thunnus* is problematical. V. North Atlantic and North Pacific subspecies of the bluefin tuna, *Thunnus thynnus*, are better considered as full species, *T. thynnus* and *T. orientalis*. VI. The yellowfin tuna, *Thunnus albacares* is a single worldwide panmictic species. VII. North Atlantic *Scomber scombrus* shows a sharp divergence from Pacific *S. australasicus* and the wide-spread anti-tropical *S. japonicus*, supporting recognition of two subgenera, *Scomber* for *S. scombrus*, and *Pneumatophorus* for the other species. VIII. Differentiation of chub mackerel supports recognition of two species, *Scomber colias* in the Atlantic and *S. japonicus* in the Indo-Pacific. IX. Nuclear and mitochondrial DNA confirms monophyly of the *Scomberomorus regalis* group of Spanish mackerels but produces a slightly different phylogeny than does morphological data.

**RÉSUMÉ.** - Maquereaux, molécules et morphologie.

La classification actuelle des Scombridae, fondée sur la morphologie, est comparée aux résultats moléculaires récents dans neuf cas. I. Le thazard bâlard, *Acanthocybium solandri*, est bien un Scombridae, et non un Xiphiodea. II. Les relations phylogénétiques des Thunnini sont confirmées, avec *Auxis*, puis *Euthynnus*, *Katsuwonus* et enfin *Thunnus* respectivement les groupes-frères des taxa restants. III. La monophylie du genre *Thunnus* est confirmée. IV. Au sein du genre *Thunnus*, la monophylie du sous-genre *Neothunnus* est confirmée, mais celle du sous-genre *Thunnus* reste problématique. V. Les sous-espèces nord-atlantique et nord-pacifique de *Thunnus thynnus* sont considérées comme des espèces distinctes: *Thunnus thynnus* et *Thunnus orientalis*. VI. Le thon albacore, *Thunnus albacares*, est une espèce panmictique. VII. Une forte divergence existe entre *Scomber scombrus* de l'Atlantique Nord, *S. australasicus* du Pacifique et *S. japonicus* à large distribution antitropicale. Ces résultats conduisent à reconnaître deux sous-genres, *Scomber* pour *S. scombrus* et *Pneumatophorus* pour les autres espèces. VIII. De la même façon, le "maquereau espagnol" comprend deux espèces: *Scomber colias* dans l'Atlantique et *S. japonicus* dans l'Indo-Pacifique. IX. L'étude de l'ADN nucléaire et mitochondrial confirme la monophylie du groupe *Scomberomorus regalis* (thazard atlantique), mais la phylogénie est légèrement différente de celle qui a été obtenue par la morphologie.

Key-words. - Scombridae, Thunnini, Tunas, Mackerels, Spanish mackerels, Taxonomy.

The currently accepted classification (Collette and Nauen, 1983; Collette *et al.*, 1984) of the mackerels and tunas (family Scombridae) within the suborder Scombroidei (Fig. 1) is largely based on classical morphological studies of Kishinouye (1923), Godsil

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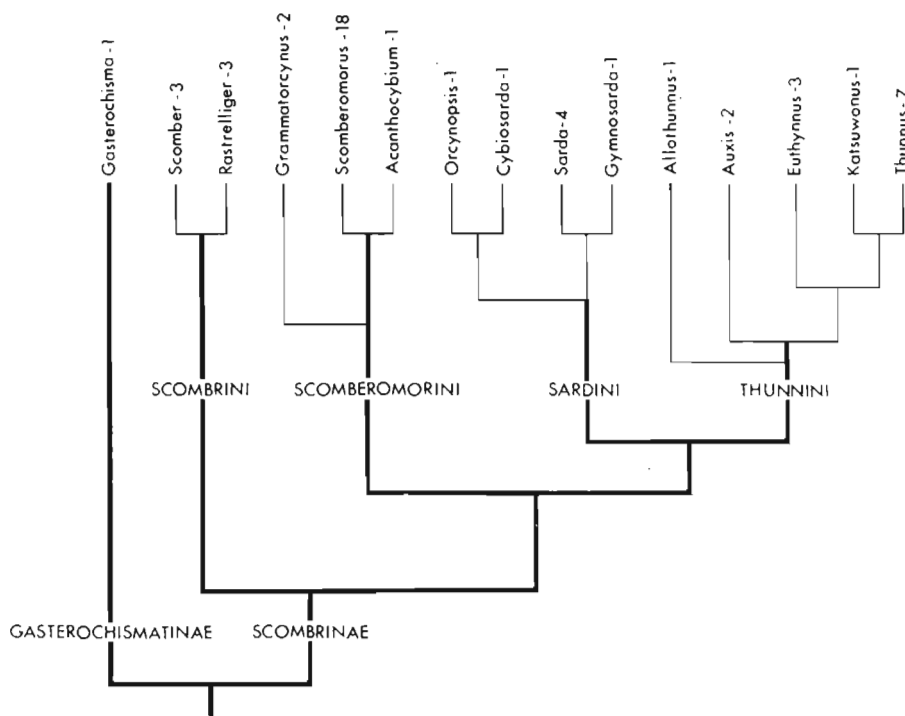


Fig. 1. - Subfamilies, tribes, and genera (with numbers of included species) of the family Scombridae. (From Collette and Russo, 1985a: fig. 1.)

and co-workers (e.g., Godsil and Byers, 1944; Godsil, 1954) and my research with a series of co-workers starting in the 1960s (e.g., Gibbs and Collette, 1967; Collette and Chao, 1975; Collette and Russo, 1985b; Carpenter *et al.*, 1995).

Recently, a number of investigators have begun to test portions of this classification with modern molecular methods using both nuclear and mitochondrial DNA (mtDNA). How well does the morphology-based classification stand up? Are changes needed in the current classification? Let us look at several recent studies and compare their molecular results with morphology-based classifications in nine cases.

I. Is the wahoo (*Acanthocybium*) more closely related to Spanish mackerels (Fig. 2B) as hypothesized by Collette *et al.* (1984) and Collette and Russo (1985a) or to the billfishes (Fig. 2A) as postulated by Johnson (1986)? A study (Finnerty and Block, 1995) of a 590 base pair region of the mitochondrial cytochrome *b* provides an hypothesis of relationships among Spanish mackerels (*Scomberomorus*), *Acanthocybium*, and billfishes (Xiphiidae and Istiophoridae). Parsimony analysis of both nucleotide and amino acid sequences places the wahoo within a monophyletic Scombridae, Gempylidae, Trichiuridae clade while the billfishes are consistently placed as a sister group to this clade and not just to *Acanthocybium*. Billfishes are genetically distant from other scombroids, perhaps constituting a separate suborder (Finnerty and Block, 1995). Parsimony analysis of the mitochondrial ATPase 6 gene (using both nucleotide and amino acid sequences) also confirms monophyly of the billfishes (Alvarado Bremer, 1994).

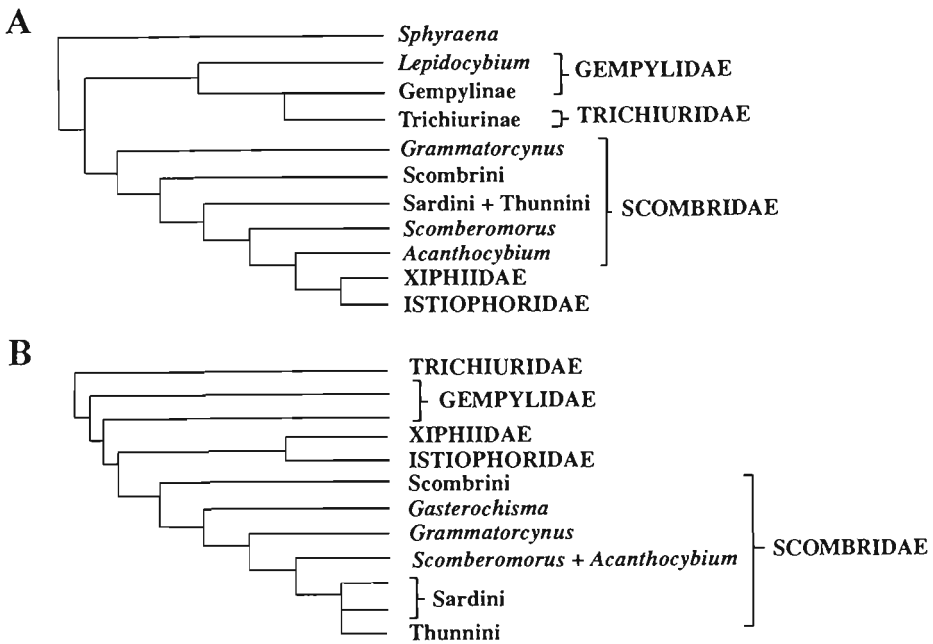


Fig. 2. - Two phylogenetic hypotheses for scombroid fishes based on morphological evidence. A: Johnson (1986). B: Collette *et al.* (1984). (From Finnerty and Block, 1995: fig. 1.)

II. Relationships within the higher tunas, Thunnini. The cytochrome *b* gene sequence provides a good resolution of relationships within the Thunnini (Finnerty and Block, 1995). Recent correspondence from Jaime Alvarado Bremer (pers. comm.) indicates that his molecular data supports the relationships of the Thunnini outlined here (Fig. 1) based on morphology, skipjack (*Katsuwonus*), is the sister-group to the tunas (*Thunnus*), then the little tunnies (*Euthynnus*), and the frigate tunas (*Auxis*). A manuscript by Graham and Dickson (pers. comm.) presents additional morphological support for the position of *Allothunnus* (a species not examined by Finnerty and Block, 1995) as the most primitive member of the Thunnini.

III. The genus *Thunnus*. Thirty years ago Gibbs and Collette (1967) published a revision of the tunas (*Thunnus*), placing the nominal genera *Parathunnus* (for bigeye), *Neothunnus* and *Semathunnus* (for yellowfin), *Germo* (for albacore), and *Kishinoella* (for longtail) in the synonymy of *Thunnus* (based on bluefin). We recognized seven species in the genus and considered the North Atlantic and North Pacific populations of bluefin tunas as subspecies, *Thunnus thynnus thynnus* and *T. t. orientalis*. Allozymes (Elliott and Ward, 1995) and the cytochrome *b* gene sequence (Finnerty and Block, 1995) provide robust support for monophyly of *Thunnus*, although material of two of the tropical species, blackfin (*T. atlanticus*) and longtail (*T. tonggol*) was not available for these studies. When all species of *Thunnus* were included (Chow and Kishino, 1995), the number of nucleotide substitutions between the outgroup, skipjack tuna (*Katsuwonus pelamis*), and species of *Thunnus* was large compared with those between species of *Thunnus*, ranging from 35-42 nucleotide substitutions (12.0-14.4%) for a 292-base pair cytochrome *b* frag-

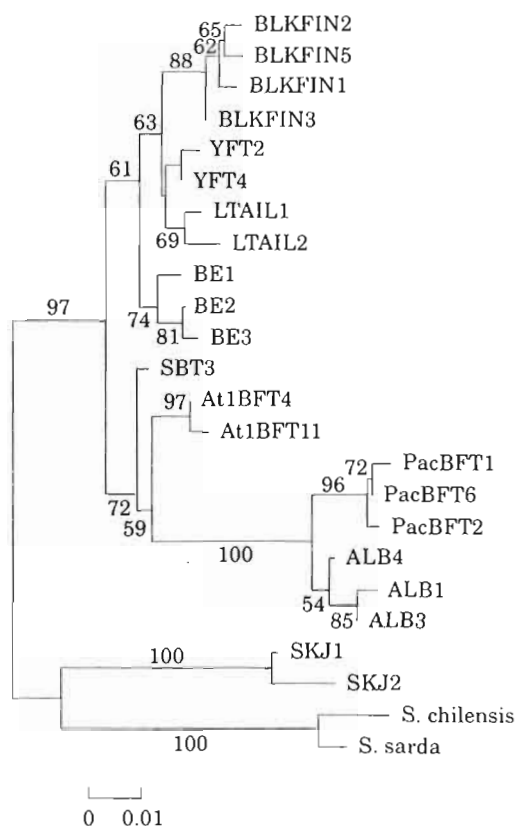


Fig. 3. - Bootstrap analysis of mtDNA data on tunas, genus *Thunnus*. Values attached to the branches are percentages of 500 bootstrap replications. BLKFIN = blackfin; YFT = yellowfin, LTAIL = longtail, BE = bigeye, SB = southern bluefin, At1BF = Atlantic bluefin, PacBF = Pacific bluefin, ALB = albacore, SKJ = skipjack. (From Alvarado Bremer *et al.*, 1997: fig. 3.)

ment and 58-69 (14.5-17.3%) for a 400-bp ATPase fragment. Between species of *Thunnus*, there were 0-10 substitutions for cytochrome *b* and 3-30 for ATPase.

IV. Subgenera of *Thunnus*. In 1979, I divided the genus into two subgenera, *Thunnus* for the coldwater clade, bluefin, southern bluefin, and albacore, and *Neothunnus* for the tropical tunas, yellowfin, blackfin, and longtail (Collette, 1979). This classification was based largely on morphological differences in the adaptations for endothermy such as liver morphology, vascularization, position of the cutaneous artery, and size of ventrolateral vertebral foramina. Species of the subgenus *Neothunnus* possess both central and lateral heat-exchangers, although the lateral retia are relatively small. Members of the subgenus *Thunnus* have their lateral retia highly developed but the central heat-exchanger has been lost or reduced and this group also has additional retia which function to elevate the temperature of their viscera, eyes, and brains. I placed the bigeye in the subgenus *Thunnus* with some hesitation because it shows some characters of both subgenera.

There seems to be little question that the members of the subgenus *Neothunnus* form a monophyletic group (Chow and Kishino, 1995; Alvarado Bremer *et al.*, 1997) but

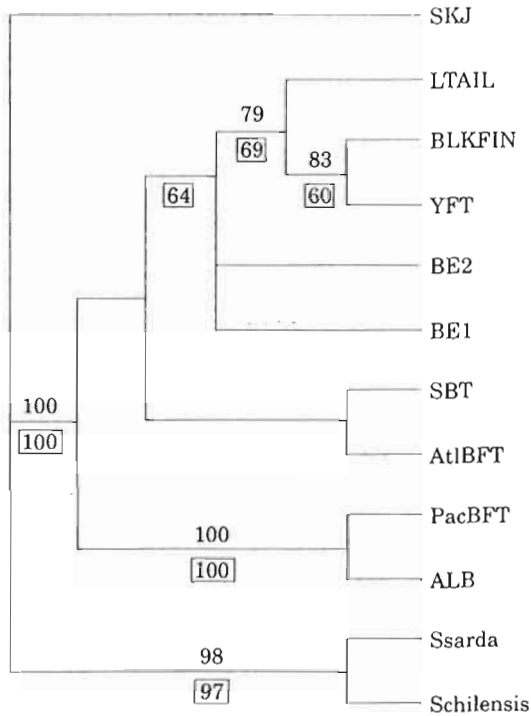


Fig. 4. - Consensus tree from bootstrap analysis using PAUP excluding four hypervariable segments of mtDNA data on tunas, genus *Thunnus*. Abbreviations as in figure 3. (From Alvarado Bremer *et al.*, 1997: fig. 4.)

there is less support for monophyly of the subgenus *Thunnus*. Several recent studies (Elliott and Ward, 1995; Finnerty and Block, 1995; Chow and Kishino, 1995) suggest that the albacore, *T. alalunga*, is the most divergent species in the genus. This has led to an alternative hypothesis that albacore and bigeye represent the most primitive species and yellowfin the most advanced (Block *et al.*, 1997). This seems unlikely, particularly since all species of the family spawn in tropical waters, and it is only the members of the subgenus *Thunnus* that have been able to extend their feeding ranges into cold waters. The most recent analysis of mtDNA from all species of *Thunnus* (Alvarado Bremer *et al.*, 1997) supports monophyly of both subgenera (Fig. 3): *Neothunnus* (BLKFIN, YFT, and LTAIL) at the top of the figure, *Thunnus* (SBT, AtlBF, PacBF, and ALB) in the middle. As with the morphological analysis, the bigeye tuna (BE) is problematical, with this tree supporting a closer relationship to the subgenus *Neothunnus* than to the subgenus *Thunnus*.

V. Species of *Thunnus*. All four studies show a surprisingly close relationship between albacore (ALB) and Pacific bluefin (PacBFT). For example, the consensus tree from bootstrap analysis using PAUP (Alvarado Bremer *et al.*, 1997) shows this relationship (Fig. 4) and also a relationship between southern bluefin (SBT) and Atlantic bluefin (AtlBFT). Chow and Kishino (1995) explain the sequence similarity between albacore and Pacific bluefin by interspecific transfer of mtDNA, with albacore mtDNA introduced by hybridization replacing most of the original mitochondrial lineages in the Pacific bluefin. Whether this is the correct explanation or not, there is a more practical problem

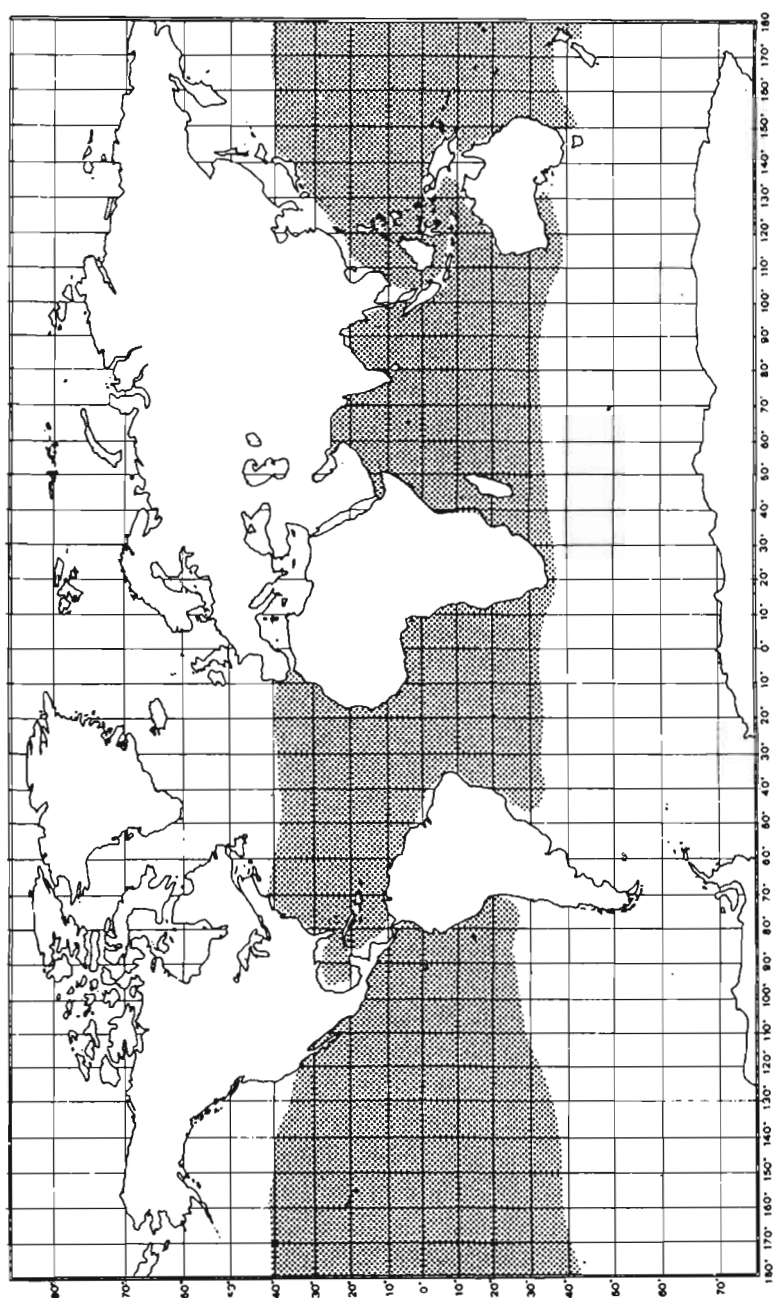


Fig. 5. - Range of the yellowfin tuna, *Thunnus albacares*. (From Collette and Nauen, 1983: p. 84.)



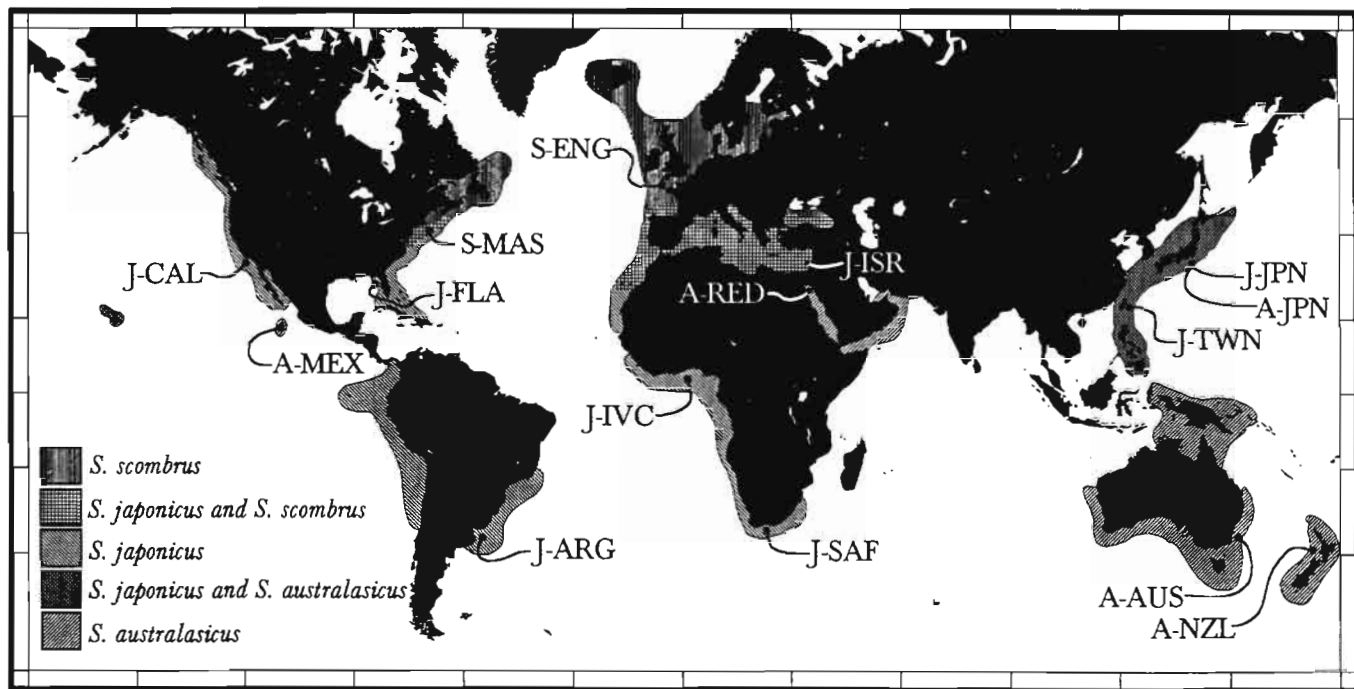


Fig. 6. - Range and sample localities of *Scomber* species. (From Scoles *et al.*, 1998: fig. 1.)

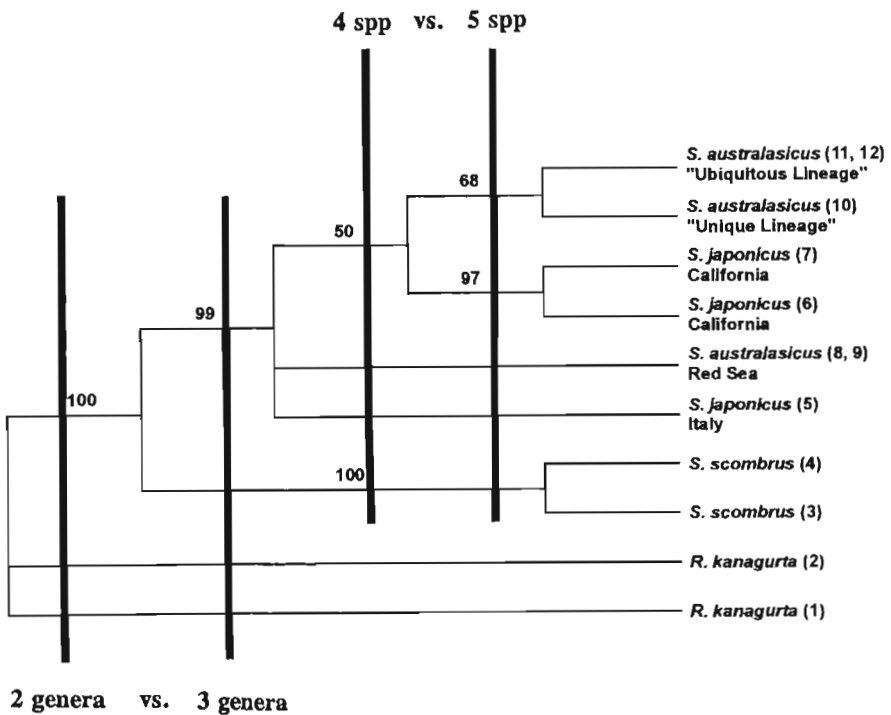


Fig. 7. - Majority rule consensus of 2 equally parsimonious trees using cytochrome *b* sequence. Numbers at nodes indicate % of times the node was supported in 1,000 bootstrap replications. (From Scoles *et al.*, 1998: fig. 5.)

involving the status of the Atlantic and Pacific bluefins. If this explanation is correct, it is a case of horizontal rather than vertical evolution and should not be the deciding factor in deciding taxonomic status. Gibbs and Collette (1967) felt that the differences between southern bluefin on the one hand (first ventrally directed parapophysis on the 9th vertebrae, yellow caudal keels) and Atlantic and Pacific bluefins on the other (first ventrally directed parapophysis on the 10th vertebrae, dark caudal keels) were best expressed by considering Atlantic and Pacific bluefins as subspecies, *T. thynnus thynnus* and *T. thynnus orientalis*, respectively. However, the morphological differences between Atlantic and Pacific bluefins (shape of the dorsal wall of the body cavity in large specimens and numbers of gill rakers) coupled with the molecular data suggests separation of these bluefins as separate species, *T. thynnus* and *T. orientalis*, rather than subspecies.

VI. Yellowfin tuna populations. The yellowfin tuna, *Thunnus albacares*, is the most wide-spread of the tropical tunas, extending around the tip of South Africa, at least during the southern summer (Fig. 5). Based on morphology, Gibbs and Collette (1967) became convinced that there was but one world-wide species of yellowfin tuna and that all 38 nominal species should be combined under the name *Thunnus albacares*. Spawning occurs across the Indian, Pacific, and Atlantic oceans in warm waters. They thought there should be significant mixing around the tip of South Africa. Scoles and Graves (1993) tested this hypothesis using mtDNA from samples of yellowfin from five Pacific and one western Atlantic localities. They found a total of 34 different genotypes but the geo-

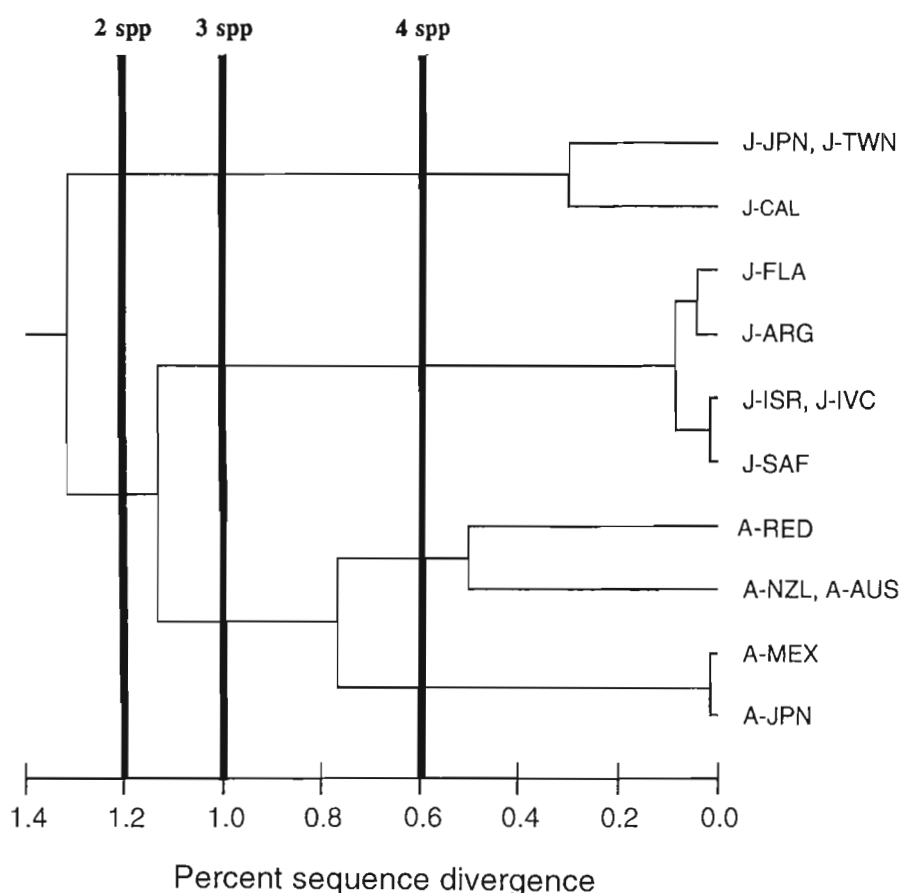


Fig. 8. - Percent nucleotide sequence divergence of populations of *Scomber japonicus* (J) and *S. australasicus* (A). (From Scoles, 1994.)

graphic distributions of the different mtDNA genotypes were similar among the six samples. Two genotypes were dominant in all samples, Pacific and Atlantic, accounting for more than half the variation. They concluded that there was sufficient gene flow between the Atlantic and Pacific to prevent the accumulation of significant genetic differentiation.

VII. Genera of mackerels, Scombrini. Currently, two genera of mackerels are recognized, *Scomber* and *Rastrelliger*, each with three species (Matsui, 1967). *Scomber scombrus* is restricted to the North Atlantic, with populations in both western and eastern North Atlantic (Fig. 6). *Scomber australasicus* has been considered as restricted to the Pacific Ocean and southeast Indian Ocean, with major populations in the northwestern Pacific - Japan, Taiwan, Philippines, and southwestern Pacific - Australia and New Zealand plus a small population in the Revillagigedo Islands off the coast of Mexico in the eastern Pacific. *Scomber japonicus* is the most wide-spread species in the genus. It is sympatric with *S. scombrus* in the North Atlantic and with *S. australasicus* in the northwestern Pacific. It is found, anti-tropically, in separate populations in all oceans. This clearly was an ideal species to compare with the wide-spread yellowfin tuna using mtDNA (Scoles *et*

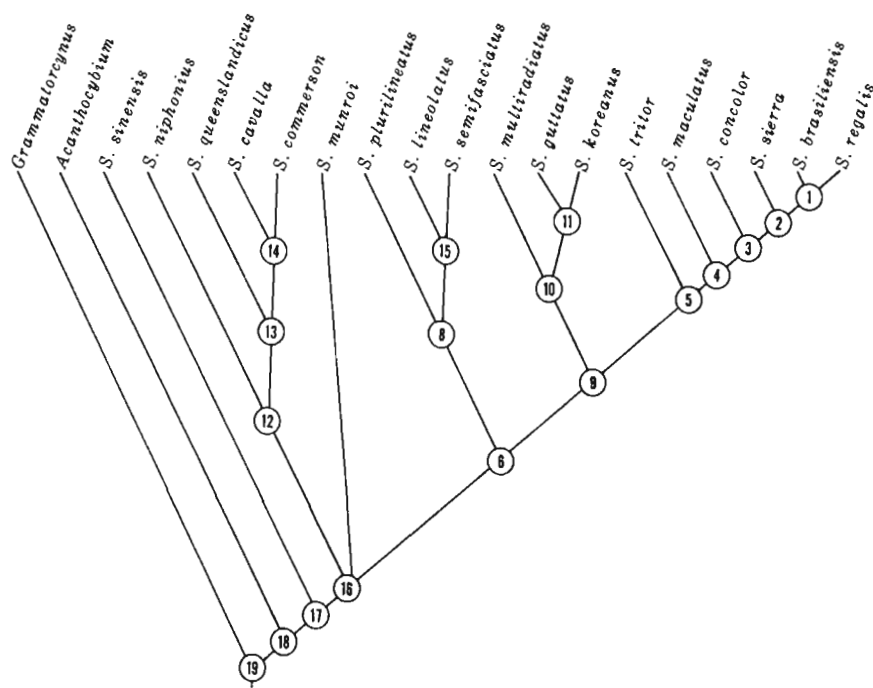


Fig. 9. - Cladogram of Spanish mackerels, *Scomberomorus* based on morphology. (From Collette and Russo, 1985b: fig. 70.)

*al.*, 1998). Most populations have been named as species or subspecies: *japonicus* Houttuyn, 1782 from Japan; *colias* Gmelin, 1789 from the eastern Atlantic; *grex* Mitchell, 1815 from the western Atlantic; *diego* Ayres, 1857 from the northeastern Pacific; *peruanus* Jordan & Hubbs, 1925 from the southeastern Pacific; and *marplatensis* Lopez, 1935 from Argentina.

How many genera of mackerels are there? A majority rule consensus of two equally parsimonious trees using cytochrome *b* with *Rastrelliger kanagurta* as the out-group shows that *Scomber scombrus* is different from *S. japonicus* and *S. australasicus* (Fig. 7). The generic name *Pneumatophorus* Jordan & Gilbert, 1883, meaning swim-bladder bearer, is available for *S. japonicus* and *S. australasicus* and has been used for them. *Scomber scombrus* has lost its swim-bladder, has 13 + 18 vertebrae instead of 14 + 17, has the first haemal spine anterior to the first interhaemal bone, has more heavily ossified bones (Matsui, 1967: table 5), and has a different otolith morphology (Fitch and Craig, 1964). Thus, both morphological and molecular data support division of *Scomber* into two genera or subgenera.

VIII. How many species of *Scomber* are there? On the right in figure 7, there are four groups: 1. *S. scombrus*; 2. *S. japonicus* from the eastern Atlantic (Italy); 3. *S. australasicus* from the Red Sea; and 4. Pacific *S. japonicus* plus *S. australasicus*. Or, moving further to the right, there are five groups, separating Pacific *S. japonicus* from the two lineages of *S. australasicus*.

Omitting *Scomber scombrus*, cluster analysis of percent nucleotide sequence divergence by restriction site analysis shows two groups at 1.2% divergence (Fig. 8), but

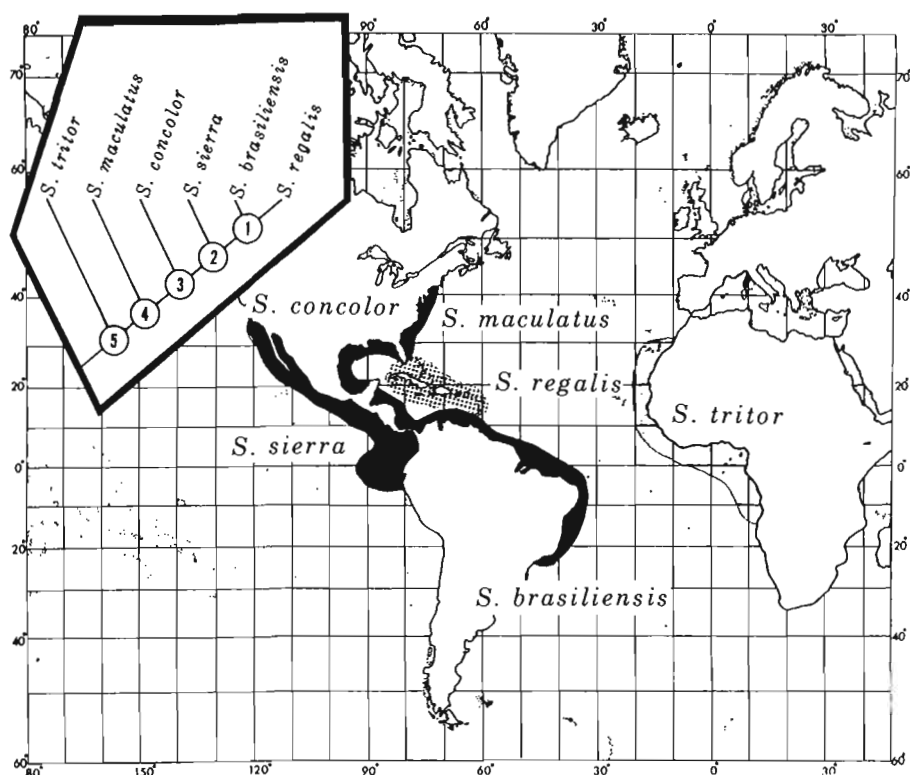


Fig. 10. - Cladogram and distribution of Spanish mackerels of the *Scomberomorus regalis* species-group.

these groups are Pacific *japonicus* (JPN, Japan; TWN, Taiwan; and CAL, California) and *australasicus* plus the Atlantic populations of *japonicus*. At 1.0% divergence, there are three groups: Pacific *japonicus*, Atlantic *japonicus* (FLA, Florida; ARG, Argentina; IRS, Israel; IVC, Ivory Coast; and SAF, South Africa) and two lineages of *australasicus*. This differentiation supports recognition of the Atlantic populations as a separate species, *Scomber colias*. There is also morphological support for this (Matsui, 1967: table 7), with Pacific populations being unmarked or only slightly marked on the belly compared to the strong markings present in Atlantic populations and the mandibular and premaxillary teeth only lightly crenulated in Pacific populations versus strongly crenulated in Atlantic populations.

When this analysis was first run (Scoles, 1994), the population of mackerels in the Red Sea was considered to be *Scomber japonicus*. The unpredicted relationships of Red Sea "*japonicus*" with *australasicus* led Erin Baker and me (Baker and Collette, 1998) to re-examine the mackerel population in the northern Indian Ocean and Red Sea which has been historically identified as *japonicus*. This population has 30-33 interneural bones under the first and second dorsal fins instead of only 26-29 and so it is NOT *japonicus* but *australasicus*, hitherto considered to be restricted to the Pacific Ocean and southeastern Indian Ocean.

IX. Spanish mackerels, *Scomberomorus*. There are 18 species of Spanish mackerels (Collette and Russo, 1985b). One well-defined monophyletic group of species within

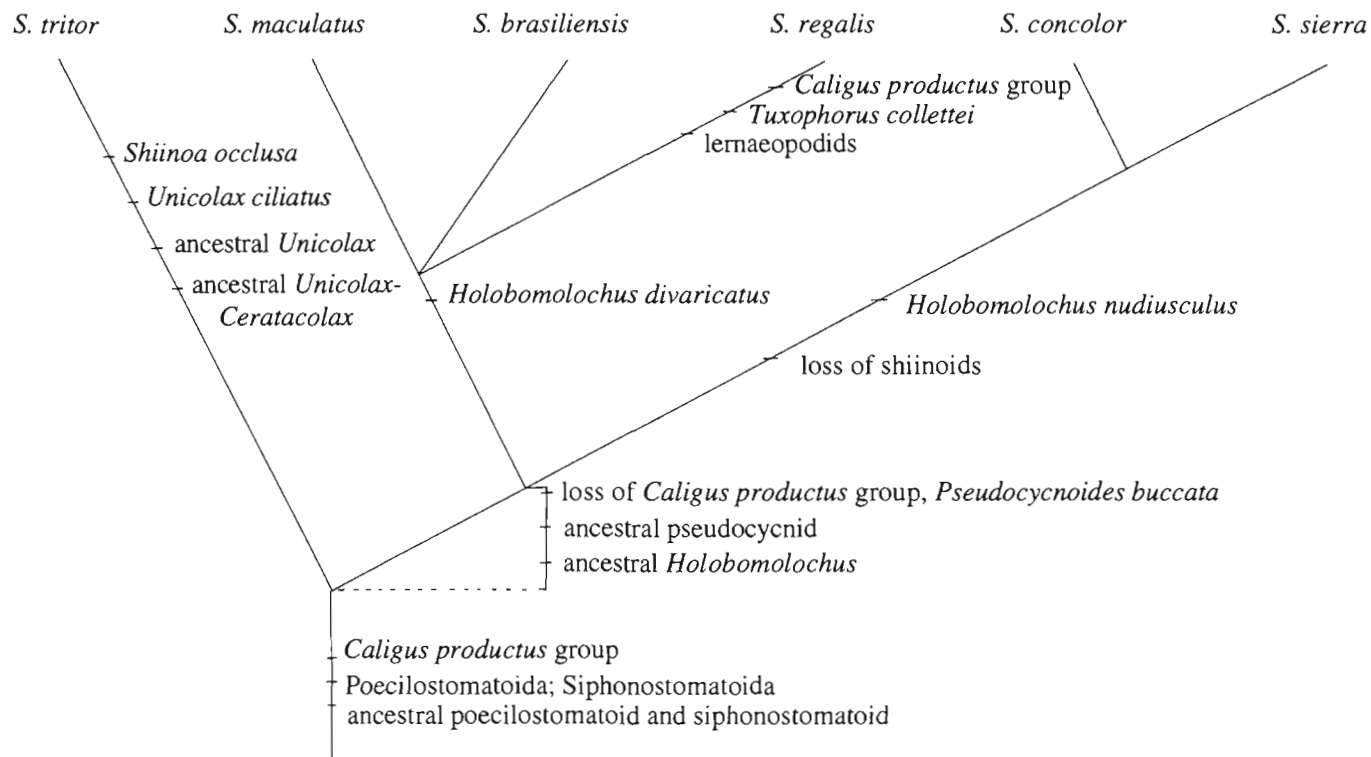


Fig. 11. - Clustering of host species of the *Scomberomorus regalis* species-group based on hypothesized parasite relationships of their parasitic copepods. (Based on Collette and Russo, 1985a: fig. 14.)

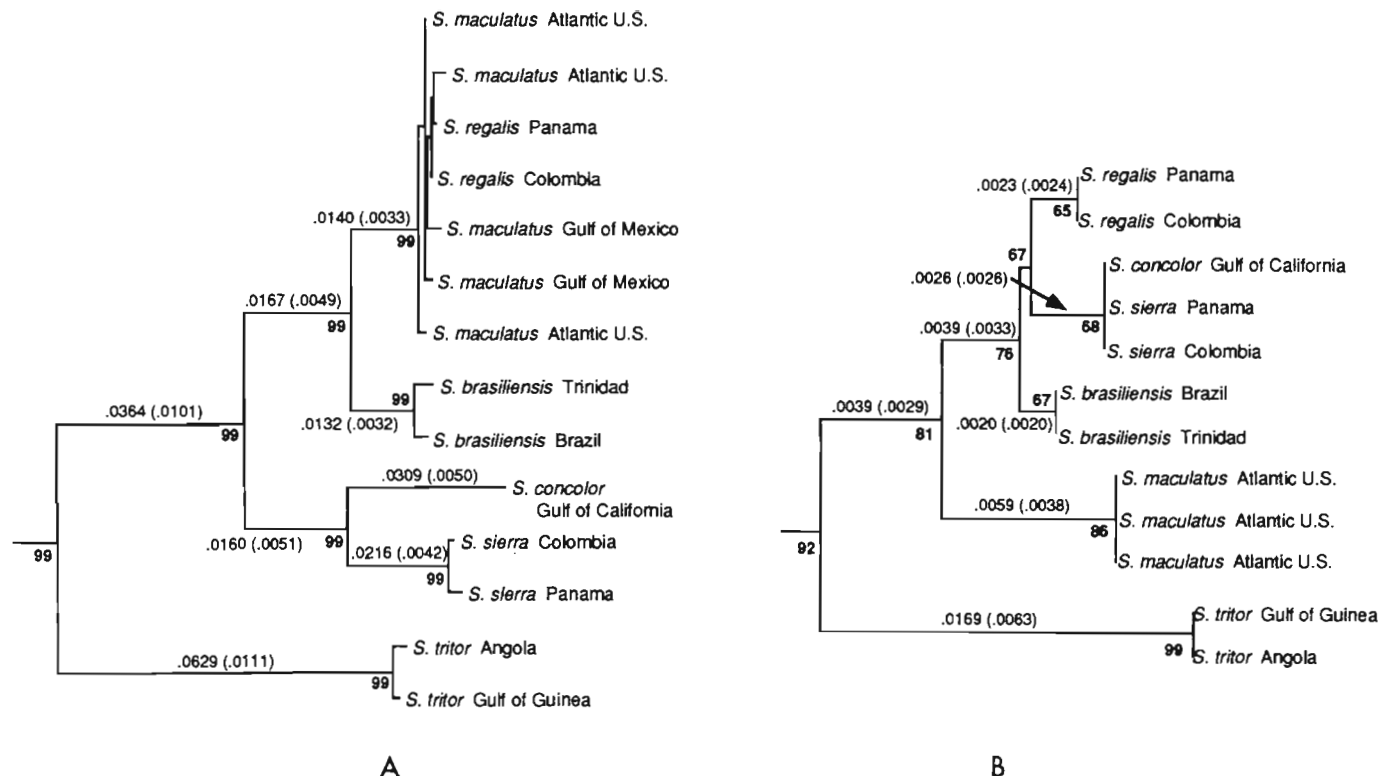


Fig. 12. - Minimum Evolution (ME) phenograms in the *Scomberomorus regalis* species-group. A. ME phenogram for combined mitochondrial genes ATP8,6 and ND2. B. ME phenogram for intron G5 in the aldolase gene *ALD*. (From Banford *et al.*, in press: figs 3A, 4A. Probability that branch lengths are greater than 0 in bold.)

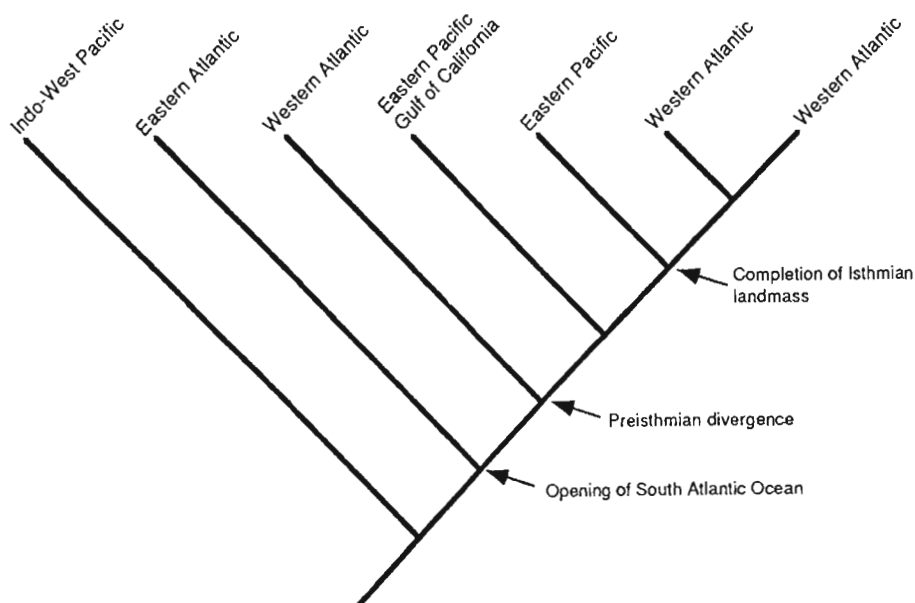


Fig. 13. - Vicariant events in the Atlantic and eastern Pacific.

the genus (Fig. 9) is the *regalis*-group. This group is defined by the presence of nasal denticles, teeth in the nose, at node 5. Characters of the anterior arterial system, pelvic girdle, and skull define nodes 1-4 (Fig. 9) within the *regalis*-group. The phylogeny constructed from morphological characters can be compared with the distributions of the species (Fig. 10).

Host-parasite relationships of parasitic copepods were then utilized to provide an independent assessment of relationships of Spanish mackerels of the *Scomberomorus regalis* species-group (Collette and Russo, 1985a). Clustering of the hosts based on hypothesized parasite relationships resulted in a slightly different tree (Fig. 11), with the two eastern Pacific species, *concolor* and *sierra* showing closer relationships than in the morphology-based tree.

In an effort to determine which tree better approximates phylogeny, Banford *et al.* (in press) re-examined the relationships of the *regalis* group using both nuclear and mitochondrial DNA with the Indo-Pacific *S. guttatus* as outgroup. The *regalis*-group is monophyletic and the Gulf of Guinea species, *S. tritor*, is confirmed as the plesiomorphic sister-species to the five American species (Fig. 12). The Atlantic Spanish mackerel, *S. maculatus*, could not be distinguished from *S. regalis* on the basis of their mtDNA sequences (Fig. 12A) due to mitochondrial introgression (Banford *et al.*, in press). Nuclear encoded aldolase sequence strongly supported the distinctiveness of *S. maculatus* and *S. regalis* (Fig. 12B), and the basal position of *S. maculatus* within the New World species. The two eastern Pacific species, *concolor* and *sierra* appear closely related to each other, more similar to the hypothesis generated from the parasite data (Fig. 11) than from that of the morphological data (Fig. 9).

Opening of the Atlantic Ocean was a major vicariant event that divided populations in the eastern Atlantic (*S. tritor*) from those in the western Atlantic (Fig. 13). The



molecular data indicate a preisthmian divergence for the western Atlantic *S. maculatus*, followed by divergence of the common ancestors of the western Atlantic (*S. brasiliensis* and *S. regalis*) and eastern Pacific (*S. concolor* and *S. sierra*).

## CONCLUSIONS

I conclude that finding a well-corroborated and stable phylogenetic hypothesis is still a process of reciprocal enlightenment. This requires careful examination of morphology, formulation of phylogenetic hypotheses, testing these hypotheses with other methodologies, such as molecular methods, and then going back to re-examine problem areas. Molecular studies will not replace morphological studies, rather they will augment them to produce a better picture of phylogeny. In all but one of the cases considered here, molecular data either support the current classification or can do so with relatively slight modification of the classification. Consideration of recent molecular data along with morphological data leads to three recommendations for changes in the current classification of the Scombridae:

1. Raise Atlantic and Pacific bluefin subspecies to species, *Thunnus thynnus* (Linnaeus, 1758) and *T. orientalis* (Temminck & Schlegel, 1844), respectively.

2. Recognize Atlantic and Pacific populations of chub mackerels as separate species, *Scomber japonicus* (Houttuyn, 1782) in the Indo-Pacific, and *S. colias* (Gmelin, 1789) in the Atlantic.

3. Recognize two subgenera within *Scomber*, the subgenus *Scomber* Linnaeus for *S. scombrus* and the subgenus *Pneumatophorus* Jordan & Gilbert for the other three species in the genus, *S. australasicus*, *S. colias*, and *S. japonicus*.

Phylogeny within *Thunnus* remains a problem with one molecular study (Alvarado Bremer *et al.*, 1997) supporting the morphological hypothesis and several other studies coming to different conclusions.

**Acknowledgements.** - I thank my colleagues for their collaboration in the morphological research that led to the morphology-based phylogeny of the Scombridae. Jaime R. Alvarado Bremer, Heidi M. Banford, J. Andrew Cooper, John E. Graves, Thomas A. Munroe, Daniel R. Scoles, and Michael Vecchione read drafts of the ms.

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**FURTHER SPECIMENS OF *AGROSTICHTHYS PARKERI*  
(TELEOSTEI: REGALECIDAE),  
WITH NATURAL HISTORY NOTES**

by

Robert M. MCDOWALL (1) & Andrew L. STEWART (2)

**ABSTRACT.** - *Agrostichthys parkeri*, originally described from New Zealand, and hitherto known from only a few specimens in the southwestern Pacific and southern Atlantic, is reported widely in the New Zealand region, including two recently beach-cast specimens. One of these, at 307 cm long, is the largest known, and was a running-ripe female collected in October; the other, probably the most complete specimen known, was 53 cm long, and had an extraordinarily elongated dorsal fin filament that extended 80% of body length. This specimen, when handled by those who discovered it still alive, appears to have emitted an electric discharge.

**RÉSUMÉ.** - Notes d'histoire naturelle sur quelques spécimens d'*Agrostichthys parkeri* (Teleostei: Regalecidae).

L'espèce *Agrostichthys parkeri*, décrite de Nouvelle-Zélande, n'était jusqu'à présent connue que par un nombre restreint de spécimens provenant du Pacifique Sud-Ouest et de l'Atlantique Sud. Parmi les nombreux individus récemment signalés dans la région, deux avaient échoué sur des plages: une femelle mature de 307 cm de long a été récoltée en octobre ainsi qu'un individu de 53 cm présentant un très long filament à la nageoire dorsale (80% de la longueur du corps). Ce dernier a été trouvé vivant et il semble qu'il ait émis une décharge électrique.

**Key-words.** - Regalecidae, *Agrostichthys parkeri*, PSE, New Zealand, Electric fishes.

The genus *Agrostichthys*, established by Phillipps (1924) for *Regalecus parkeri* Benham, 1904, was first recorded from a specimen beached at Deborah Bay, southern New Zealand, and is known in New Zealand as ribbonfish (Paulin *et al.*, 1989), though it was called streamerfish by Nelson (1994). It belongs to the lampridiform family Regalecidae, which comprises just two apparently monotypic genera of highly aberrant, extremely slender, compressed, and very elongated fishes: *Regalecus glesne* and *Agrostichthys parkeri* (see Walters and Fitch, 1960). *R. glesne* is perhaps the longest known teleost, reportedly reaching lengths exceeding 17 m and weights of over 300 kg, and is attributed with generating stories of "sea serpents" (Dybas, 1993). *A. parkeri* appears to be much smaller, but reaches at least 307 cm TL. The biology of both species is poorly known, particularly *A. parkeri* about which virtually nothing has been written. In addition to his holotype, Benham (1904) reported a specimen of *A. parkeri* from Stewart Island, at the southern end of New Zealand. Phillipps (1924) obtained a third specimen, beached at

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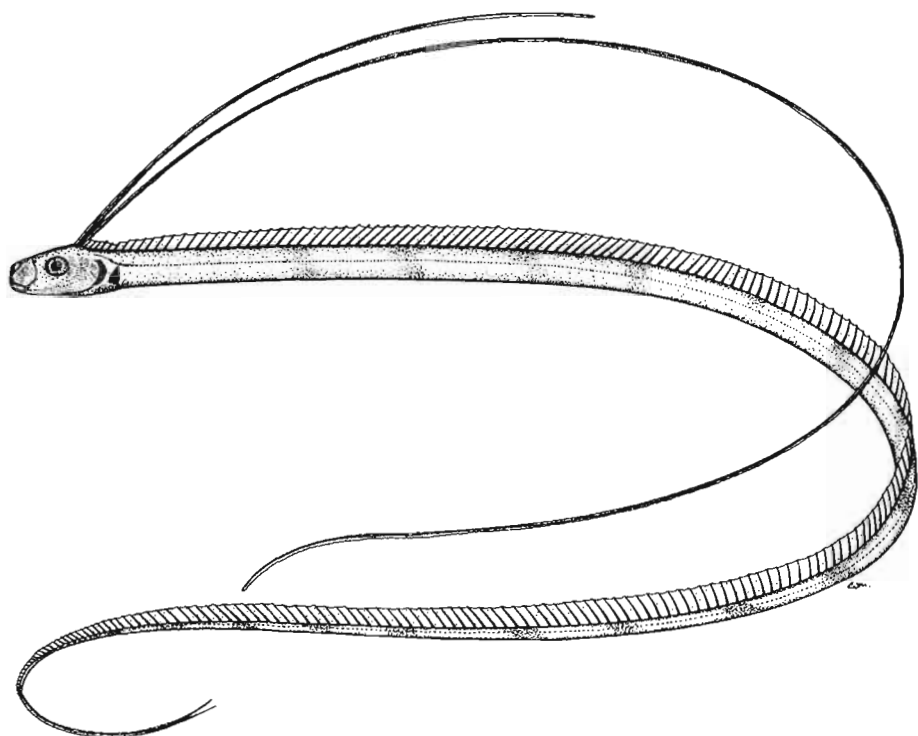


Fig. 1. - *Agrostichthys parkeri*, drawing based primarily on the specimen 530 mm TL, NMNZ P. 34476, beach-cast at Kauaaurau Bay, Pelorus Sound, July 1995 (drawn by Erica Mackay, from preserved specimen, but restoring the length of the dorsal fin filament to that prior to shrinkage during preservation, with detail augmented by another specimen in Museum of New Zealand collections, of similar size).

Cape Palliser on the southern coast of the North Island, New Zealand, only the head and caudal regions of which were retained. Scott (1934) described *A. benhami* from Tasmania, but this was provisionally treated as a synonym of *A. parkeri* by Paxton *et al.* (1989) and, presumably as a result, Nelson (1994) described the genus as monotypic. Trunov (1982) discussed a further three specimens from the southern Atlantic, one of which was near complete, the others comprising only the head and anterior trunk. Heemstra (1986) knew of "7 known specimens... only 1 intact"; Olney *et al.* (1993) refer to a further specimen from the collections of the Institut für Seefischerei, Universität Hamburg, Germany. These seem to comprise all of the few published records of *A. parkeri*. Gomon *et al.* (1994) knew of no records from the southern coastal seas of the Australian mainland, and the only Australian record is that by Scott (1934) from Tasmania (according to Paxton *et al.*, 1989). Bauchot (1984) mentioned it as not occurring in the western Indian Ocean, but she surmised that it may be present there, though perhaps rare. Thus, there have been very few published reports of *A. parkeri*, and few of the fish taken have been intact, though there seems an expectation that it may be widespread.

This note is prompted by recent findings of two further nearly intact specimens, and discusses numerous additional specimens in the holdings of the Museum of New Zealand.

### MATERIAL EXAMINED

A specimen of *A. parkeri* was found in the shallows of the sea in Kauauro Bay, Marlborough Sounds, at the northern tip of New Zealand's South Island, in July, 1995 by Jason McKenzie (Animal Sciences Department, Lincoln University, Christchurch, New Zealand). Kauauro Bay is towards the outer end of Pelorus Sound, and although an inside-facing embayment within the sound, is close to the open sea of Cook Strait. The fish was alive when found, though moving only weakly. The specimen (Fig. 1, Museum of New Zealand no. P. 34476) is nearly intact, and is 53 cm long—the caudal peduncle tapers to a very fine tip, so that it seems unlikely that much has been lost apart, perhaps, from the caudal fin itself. The other specimen was beached, alive, at Napier, on the mid-east coast of the North Island, and was recovered by Clinton Duffy of the Department of Conservation, on 6 October, 1993 (NMNZ P. 30830 - only the head and caudal regions, and ovaries, were preserved). It was still alive, though weakened probably through prolonged rolling in the surf before being retrieved. It also was nearly intact, though it retained no elongated rays in the dorsal fin and there is no caudal fin, only a well-healed stump (but see below).

In view of the paucity of records, the opportunity is taken to report 40 additional specimens in the fish collection of the Museum of New Zealand. Most of them were trawled, but eight adults and one juvenile were retrieved as beached (the adult specimens often broken and partly eaten by sea birds). Two specimens were speared by scuba divers in shallow water, one of which was reported to have been swimming strongly amongst kelp.

### *AGROSTICHTHYS PARKERI* (BENHAM, 1904)

#### Identification and characteristics

We refrain from detailed redescription of the species since previous authors (Benham, 1904; Phillipps, 1924; Scott, 1934 - especially; Trunov, 1982) describe their specimens, at length and in detail. However, some features of the latest specimens warrant detailed discussion, especially where they differ from previous accounts.

The specimens key to family Regalecidae in Paulin *et al.* (1989), and conform to previous descriptions of *Agrostichthys parkeri* in most respects (Benham, 1904; Phillipps, 1924; Scott, 1934). *A. parkeri* is greatly compressed as well as extraordinarily elongated (Pl. 1, Fig. 1). The body depth of the Kauauro Bay specimen, which is greatest immediately behind the head, is c. 11 mm, or < 2.1% of body length (taken to the tip of the specimen, this distance referred to hereafter as "body length" owing to uncertainty that it equates to standard length). The body tapers slowly to almost a point at the posterior tip. The anus is well forward on the trunk (length to vent 150 mm, or c. 28% of body length). The mouth is oblique with much enlarged flattened maxillae overlying the extremely protrusible mouth. There are no scales, but the body is covered with small almost horny nodules in irregular, longitudinal rows (see Pl. 1A, immediately behind head). The lateral line follows the ventral body profile for most of the body length.

The dorsal fin originates above the head a little behind the eyes and is in two parts—anteriorly a small triangular fin and behind it a very long low fin with many rays of about equal length. The first and second rays in the dorsal fin of the Kauaurau Bay specimen, especially the second, are greatly elongated, extending as single, free filaments. These filaments shrank considerably following preservation (Jason McKenzie, pers. comm.). The specimen was preserved in methanol, the only available preservative at the remote location where it was found, which resulted in considerable dehydration and shrinkage of the dorsal fin filaments. However, their length in life can be estimated from a photograph of the fish taken by Mr. McKenzie. From this we estimate that the second filament was naturally 466 mm long, or 88% of body length (Fig. 1). In preservative it is c. 376 mm long, indicating shrinkage of 20%. The first ray in the dorsal fin which is separate from the second ray along most of its length, measures about 125 mm in preservative. If we assume similar shrinkage to that indicated above for the second ray, the first may originally have been c. 16 cm long, or 30% of body length and thus longer than the single filament illustrated by Trunov (1982), which we estimate as about 20% of body length. This difference between the Kauaurau Bay specimen, and Trunov's illustrated specimen seems unlikely to be due to allometric growth, since Trunov's specimen was 579 mm SL, and thus little longer than ours. We conclude that Trunov's specimen was probably incomplete (note that two of his three specimens were so badly mutilated in capture that only the head and anterior trunk were recovered—it seems that his capture techniques were damaging to small, delicate fishes). As most of the Museum of New Zealand juvenile specimens were trawled, they also have been damaged, including breaking and loss of the elongated rays.

The Kauaurau Bay specimen is unique in being apparently complete, apart from the lack of a caudal fin. Most large specimens in the Museum of New Zealand's collections of *A. parkeri*, including the very large example found at Napier, have no caudal fin, but just an apparently well-healed stub (Pl. 1). A radiograph of partial specimen NMNZ P503 shows that the vertebral column ends in a complete vertebra with no hypural skeleton, in this instance confirming loss of part of the caudal region. Judging by specimens available, this sort of damage may be common, both in nature, and when specimens are captured. One specimen about 70 cm long (NMNZ P. 13400 - taken on the southern slopes of the Chatham Rise, in a high-opening bottom trawl fishing at > 900 m depth) has two greatly elongated caudal filaments, one 55 mm the other 75 mm long; there is, of course, no assurance that these filaments are complete.

Both elongated dorsal fin rays have paired rows of uniform, tiny, hard, closely-spaced spinules along the postero-lateral edges of the proximal part of the rays. These spinules seem to disappear distally, where the rays are invested in loose skin which has shrunk onto the fin ray within, on preservation. Before preservation, therefore, the filaments may have been several millimetres in diameter with a relatively significant space between the fin ray and the surrounding skin that is now shrunk onto the fin ray. There is no anal fin. Pelvic fins are reduced to short spikes that may have been attached to the abdomen by membrane, but their original form is uncertain, and they may have been badly eroded or damaged.

Trunov (1982) mentioned a "dermal protuberance in the form of an uvula" beneath the dentary symphysis. This description is apt for specimens of *A. parkeri* in collections of the Museum of New Zealand, including the Kauaurau Bay and Napier beached fishes, in which the structure is quite large and obvious, being darkly pigmented. With the mouth closed it lies within the lateral covering of the dentaries, and is attached to the skin along

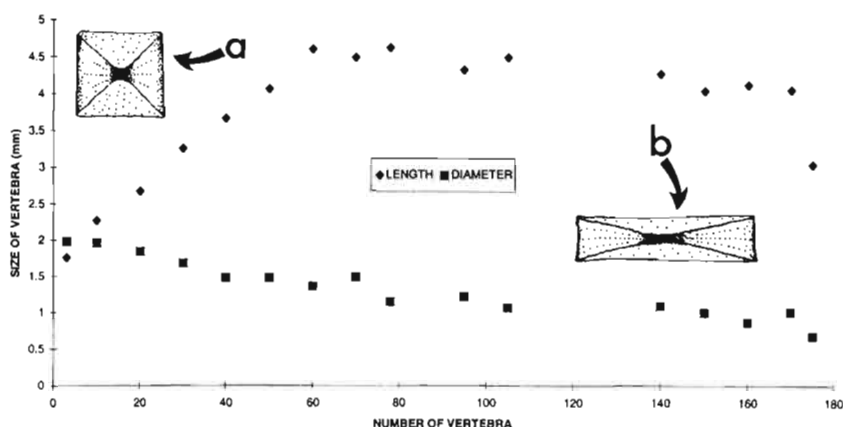


Fig. 2. - Change in shape of vertebrae in *Agrostichthys parkeri*, derived from radiograph of specimen NMNZ P 13400 (figures of vertebrae, drawn from the radiograph, are inset at the approximately appropriate distance along vertebral column - vertebrae # 3 and # 140).

most of its length. The mouth of *A. parkeri* is clearly highly protrusible (Pl. 1B) and, on full extension, forms an elongated and tubular opening with distinct antero-ventral orientation. This results from only the upper jaw protruding, such that, in plate 1C, the distance between the eye and the tip of the lower jaw does not change with protrusion.

Vertebral count is estimated at c. 175-180 (from two fish, NMNZ P13340 and P 25012). These are best estimates from radiographs which were difficult to read in places, undoubtedly owing to light ossification in these fish, and this was not helped by the fact that the fish were fragmented. Of some interest is the fact that the shape of the vertebrae changes markedly along the length of the fish. Vertebra length and diameter were determined using a digital display micrometer that reads to 0.01 mm, under a dissecting microscope. Just behind the head (Fig. 2), vertebrae are about as long as their diameter (see arrowed inset a), but both size and proportions change, with diameter declining steadily along the vertebral column while length increases markedly up to about vertebra 50 before beginning a steady decline in vertebra length towards the tail; these changes result in mid-length to posterior vertebrae becoming shaped like slender spindles (Fig. 2, arrowed inset a).

### Colouration

When encountered alive, the Kauaurau Bay specimen's body was shimmering silver, with indistinct vertical dusky bands, these most intensely coloured on the back and belly, fading mid-laterally. The longest dorsal fin filament was bright pink, interrupted by numerous dark bands. In preservative the fish is white with about 14 grey/black hour-glass-shaped bands across sides, darkest and broadest at dorsal and ventral body profiles, paling and narrowing towards mid-lateral trunk. The large specimen beach-cast at Napier was also bright silver, with indistinct dusky grey banding, much of the head reddish, and the fringes of the long, low dorsal fin dusky.

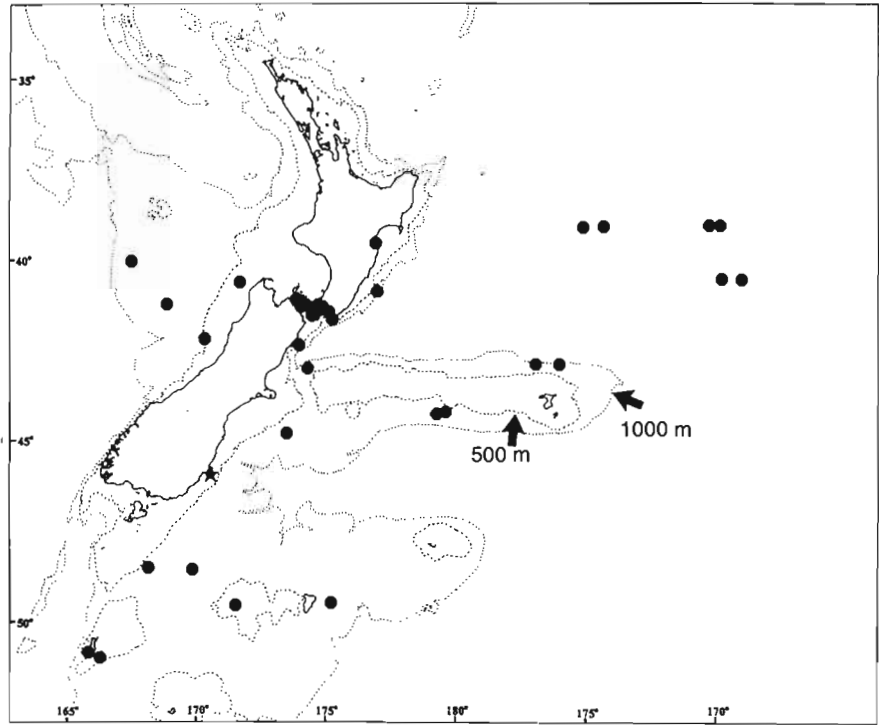


Fig. 3. - Distribution of *Agrostichthys parkeri* in the New Zealand region (• Museum of New Zealand records; \* type locality), with 500 and 1000 m contour lines. Some points represent more than one specimen.

### Size

Although not reaching the size of *Regalecus glesne*, *Agrostichthys parkeri* nevertheless reaches a considerable length. One of Benham's (1904) specimens was 191 cm long, while Phillipps' (1924) measured more than 274 cm. Scott's (1934) specimen measured 81 cm. The six largest examples in the Museum of New Zealand collections are 246-307 cm TL, the largest being that beach-cast at Napier.

### Distribution

*A. parkeri* previously has been recorded from around New Zealand, off northern Tasmania (Australia; Scott, 1934) and in the southeastern Atlantic (Trunov, 1982). There is a clear, but still unfulfilled, expectation that the species will be found to occur more widely (Bauchot, 1984; Heemstra, 1986; Paxton *et al.*, 1989; Gomon *et al.*, 1994). New Zealand specimens have been taken within or south of the sub-tropical convergence (Fig. 3), and mostly since about 1980. The northernmost records are from around 39°N, off the east coast of the North Island of New Zealand. The southernmost records are six adults from the Campbell Plateau, including one specimen beached on Adams Island, the southernmost of the Auckland Islands (Fig. 3). No juveniles have been taken south of 42°S. Eleven records are from the Cook Strait/Wellington region, possibly reflecting its proximity to deep water and the vigorous ocean currents moving through the strait. Captures around New Zealand are associated partly with research mid-water trawling by the



former Fisheries Research Division of the New Zealand Ministry of Agriculture and Fisheries (now part of the National Institute of Water and Atmospheric Research), e.g. on one research cruise in March 1995 the GRV *Tangaroa* took six juveniles (< 250 mm TL) in fine mesh trawls fishing between 12 and 108 m, over 4,350–5,000 m; more frequent captures also relate partly to the intensive development of deep-water commercial fisheries around the region.

The New Zealand captures indicate that, at least in the southwestern Pacific Ocean, *A. parkeri* is widespread and perhaps not uncommon. Specimens captured by trawling typically were taken in water of moderate to great depths, often over 500 m and up to 5,000 m, but sometimes as shallow as 100 m or less. Possibly it is a pelagic mid/surface water species, unaffected by the actual water depth over which it is living.

### Natural history notes

The small Kauauro Bay specimen showed no obvious signs of gonad development and may be immature. The very large Napier specimen, however, was shedding ovulated eggs freely when recovered, and so was clearly ripe. The eggs were a pale amber colour and about 4 mm in diameter. A capture date of 6 October indicates spring spawning.

How protrusion of the mouth is exploited in feeding is not known. Typically a down-turned mouth opening is indicative of bottom-feeding (Keast and Webb, 1966; Wootton, 1991), but this seems highly implausible for *A. parkeri* given its usual capture at upper-mid depths over often deep water. Possibly, the fish hangs obliquely in the water column so that the margins of the protruded mouth opening provide a vertical opening for engulfing food. The mechanics of protrusion, in terms of Motta's (1984) classification, are unknown and a likely source of interest, especially given the apparently unusual distinctly antero-ventral orientation of the oral aperture, relative to the long body axis of the fish, after protrusion.

## DISCUSSION

Jason McKenzie reported that when he picked up the fish, and was holding it by the caudal end with the dorsal fin filament draped over his hand, the fish wriggling gently, he experienced what he described as mild but quite distinct pulsed electric shocks that passed up through his hand into the forearm at intervals of about 10 s. A companion who independently held the fish reported the same sensation. Whether the sensation was truly an electrical discharge needs to be confirmed by use of appropriate electrical testing equipment should specimens be available. However, given that the fish is so rarely collected and, when collected, is usually dead and/or badly damaged, this confirmation is going to depend heavily on chance. Whether the shocks experienced the collectors of the Kauauro Bay fish were of reduced intensity as a consequence of the fish's enfeebled condition is unknown but this seems likely (trawl-caught electric rays are observed to have discharged their electric capacity during the trauma of capture), but no electric discharges by the Napier specimen were reported, although it was also handled alive.

Electric discharges have long been recognised or suggested for a variety of fish groups, including several marine skates and rays (Rajidae and Torpedinidae: Rajiformes), the African freshwater elephant fishes (Mormyridae) and gymnarchids (Gymnarchidae: Osteoglossiformes), South American electrophorid electric eels (Electrophoridae, Sternopygidae and Rhamphichthyidae: Gymnotiformes), the electric catfishes (Malapteru-

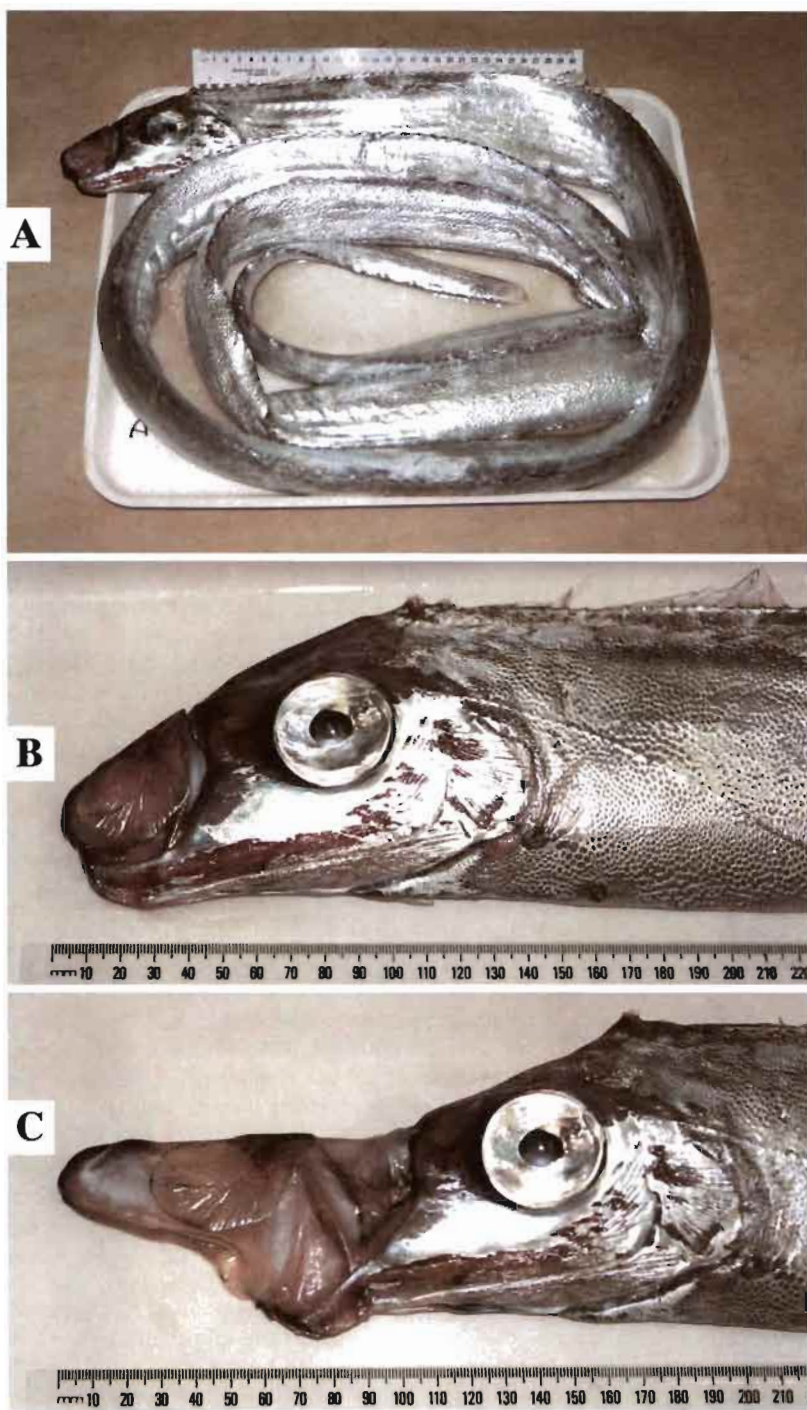


Plate I. - A: *Agrostichthys parkeri*, 307 mm TL, from Napier, 6 October 1993 (NMNZ P.30830); B: Head with mouth closed; C: Mouth protruding (photos: Clinton Duffy).

ridae: Siluriformes), and a few species of marine stargazers (Uranoscopidae: Perciformes) (Bennett, 1971; Marshall, 1971; Moller, 1995). More recently another catfish family (Mochokidae) has been described as weakly electric (Hagedorn *et al.*, 1990). The ability to generate electricity has thus clearly evolved several times within divergent groups of cartilaginous and bony fishes.

There have not to our knowledge, however, been suggestions that any of the morphologically bizarre fishes of the Order Lampridiformes have this ability. *A. parkeri* has been collected so rarely, and when collected is so often dead and/or badly mutilated during capture, that it is perhaps not surprising that its apparent ability to generate electricity has not hitherto been reported. The nature of the phenomenon, the origin of any electric discharges, and their function, are at present matters only for speculation, pending the recording of an electrical discharge and anatomical investigations that may identify where any electric current originates. The peculiar, uvula-like structure beneath the lower jaw may be implicated. The reports of mild shocks experienced by the individuals who collected the Kauaerua Bay fish, implicate the tail region of the fish and the extraordinarily elongated dorsal fin filament, since these are the parts of the fish with which they were in direct contact.

Bennett (1971) distinguished between strong and weak electrical discharges. The former he presumed to function in either defence or in securing prey, while the latter function as a part of the electrosensory system, enabling the fish to detect objects by the distortion they cause in the field established by the electric organ. If the elongated dorsal fin filament is implicated in generating a field, it could perhaps be some form of "electric fishing" organ for securing prey. This perhaps seems bizarre, but fish have an extraordinary ability to evolve structures and habits that almost defy the human imagination. Marshall (1971) speculated that fish that use electric discharges to sense prey or predators may need to keep the receiving organ (lateral line) from undulating laterally while swimming (hence gymnotids swim rippling the elongated anal fin). If this should be true of *Agrostichthys*, perhaps its elongated filament provides this capacity by streaming behind the fish while its body does undulate during swimming. A live oarfish, *Regalecus glesne*, has been observed swimming, perhaps hanging, vertically, undulating the long dorsal fin for propulsion (C.D. Paulin, Museum of New Zealand, pers. obs., 1979). A photograph of an oarfish positioned in vertical orientation was recently published (National Geographic, December 1996), in which the elongated dorsal fin rays were splayed vertically in front (above) the snout of the fish, and the elongated pelvic rays projected laterally. Fishes of the related lampridiform family Trachipteridae have also been recorded oriented vertically with elongated fin rays displayed in this fashion (Heemstra and Kannemeyer, 1984). Possibly, *A. parkeri* also adopts a similar vertical orientation, but where its extremely elongated dorsal fin filaments project is unknown.

Alternatively, electric discharges may be some kind of protective mechanism for a far from robust fish. Greatly elongated fin filaments are present in other lampridiform fishes of several families, variously involving the dorsal and/or pelvic and/or caudal fins (Walters and Fitch, 1960; Nelson, 1994; Stewart, 1995); these are of unknown function and we suggest that examination of other lampridiform fishes for the ability to generate electric current might be worthwhile.

**Acknowledgements.** - We thank Jason McKenzie for returning the specimen found at Kauaerua Bay, and for his observations on the electrical discharge; Clinton Duffy, Department of Conservation, Gisborne, for photographs and observations of the Napier specimen, and Chris Paulin, Museum of New

Zealand, for observations on swimming oarfish; Gavin James, NIWA, Christchurch, Phil Heemstra, JLB Smith Institute of Ichthyology, Grahamstown, South Africa, and Bernd Kramer, Zoologisches Institut, Universität Regensburg, Regensburg, Germany, for reviewing the manuscript. This work was supported (AS) by New Zealand Foundation for Research, Science and Technology contract CO MNZ603 to Museum of New Zealand Te Papa Tongarewa. Robin McPhee, Museum of New Zealand took the radiographs.

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## Symposium 4

### DEEP-SEA FISHES

Chairperson: **Tomio IWAMOTO**

- AKAZAKI M. & B. SÉRET. - *Dentex fourmanoiri*, a new species of sea bream 177-183  
(Sparidae: Denticinae) from off New Caledonia.  
*Dentex fourmanoiri, une espèce nouvelle de denté (Sparidae, Denticinae) de Nouvelle-Calédonie.*
- DUHAMEL G. - Deep-sea demersal ichthyofauna off the St-Paul and Amsterdam 185-194  
Islands (central Southern Indian Ocean).  
*L'ichthyofaune démersale profonde des îles St-Paul et Amsterdam (Océan Indien Sud).*
- ROBERTS C.D. & T.C. GRANDE. - The sandfish, *Gonorynchus forsteri*, 195-205  
(Gonorhynchidae) from bathyal depths off New Caledonia, with notes on New Zealand specimens.  
*Le "sandfish", Gonorynchus forsteri (Gonorynchidae) des eaux profondes de Nouvelle-Calédonie et remarques sur des spécimens de Nouvelle-Zélande.*



## **DENTEX FOURMANOIRI, A NEW SPECIES OF SEA BREAM (SPARIDAE: DENTICINAE) FROM OFF NEW CALEDONIA**

by

Masato AKAZAKI (1) & Bernard SÉRET (2)

**ABSTRACT.** - A new species of sea bream, *Dentex fourmanoiri* is described from 16 specimens caught in deep water off New Caledonia. It is distinguishable from other species of *Dentex* by the number of dorsal fin rays (XIII + 9), a more acute snout angle, a greater eye, and the bright yellow margins of the dorsal and caudal fins.

**RÉSUMÉ.** - *Dentex fourmanoiri*, une espèce nouvelle de denté (Sparidae, Denticinae) de Nouvelle-Calédonie.

Une nouvelle espèce de denté, *Dentex fourmanoiri* est décrite à partir de 16 spécimens récoltés dans les eaux profondes de Nouvelle-Calédonie. Elle diffère des autres espèces de *Dentex* par le nombre de rayons à la nageoire dorsale (XIII + 9), un museau plus aigu, un oeil plus grand, et les bords des nageoires dorsales et caudale jaune vif.

**Key-words.** - Sparidae, Denticinae, *Dentex fourmanoiri*, ISEW, New Caledonia, New species, Taxonomy.

The genus *Dentex* includes 10 species but there are only two recorded species of yellow sea bream in the West Pacific, both known from Japan (Akazaki in Masuda *et al.*, 1984). These are *Dentex* sp. and *Dentex tumifrons* (Temminck and Schlegel, 1843) originally described from Nagasaki, Japan. The latter is also reported from north-western Australia (Gloerfelt-Tarp and Kailola, 1984; Sainsbury *et al.*, 1985), and from the South China Sea (Lee, 1983).

In the 1970s, P. Fourmanoir, former ichthyologist in ORSTOM (Institut de recherche scientifique pour le développement en coopération), collected a specimen of a probable new species of a yellow sea bream from off New Caledonia, and sent it to the first author (MA) who reported on this discovery during the XIth annual meeting of the Ichthyological Society of Japan (21 March - 1st April, 1978), as mentioned in vol. 25, n° 1 of the Japanese Journal of Ichthyology (p. 75). Unfortunately, the specimen was lost and the description could not be completed.

In the course of the exploratory cruises performed by ORSTOM in the economic zone of New Caledonia to investigate the bathyal fauna and new resources (Séret, 1997), 16 specimens of this new sea bream were collected and are herein described.

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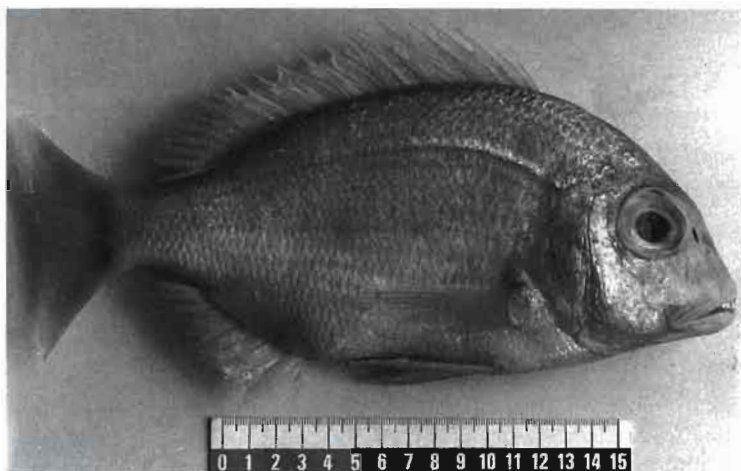


Fig. 1. - Holotype of *Dentex fourmanoiri* n. sp. (MNHN 1998-301), 226 mm SL, from Chesterfield Islands.



Fig. 2. - Paratype of *Dentex fourmanoiri* n. sp. (MNHN 1998-302), 178 mm SL, from northern New Caledonia.

## MATERIALS AND METHODS

The 16 specimens of the new species are listed in the type series below. The new species was compared to closely related species: 42 specimens of *Dentex tumifrons* from Japan and East and South China seas, and 42 specimens of *Dentex* sp. from Okinawa and the Ogasawara Islands.

Methods for taking measurements and meristic data follow Akazaki (1962); counts were made on radiographs. An analysis of covariance was done on selected morphometric ratios.

The specimens are deposited in the Muséum national d'histoire naturelle, Paris (MNHN), the National Science Museum, Tokyo (NSMT) and Miyazaki University (MU).



**DENTEX FOURMANOIRI N. SP.**

(Figs 1, 2, Table I)

**Type series**

*Holotype*. - MNHN 1998-301, 1 specimen (226 mm SL), R.V. "Coriolis", CORAIL 2, CP 130, 19°27'S, 158°34'E (east of Chesterfield Islands), beam trawl, 217 m depth, Jul. 1988.

*Paratypes*. - MNHN 1997-4140, 3 specimens (164-190-215 mm SL), R.V. "Coriolis", CORAIL 2, stn. 131, 19°25'S, 158°37'E (east of Chesterfields Islands), beam trawl, 217 m depth, 29 Aug. 1988; MNHN 1997-4141, 2 spms (103-190 mm SL), R.V. "Alis", BATHUS 1, stn. 712, 21°44'S, 166°35'E, beam trawl, 210 m depth, 9 Mar. 1993, col. B. Richer de Forges; MNHN 1997-4142, 2 spms (190-205 mm SL), R.V. "Alis" BERYX 4, stn. 3, 22°44'S, 167°17'E, longline, 260 m depth, 23 Jan. 1992, col. R. Grandperrin; MNHN 1998-302, 5 spms (113-178 mm SL), R.V. "Vauban", MUSORSTOM 4, stn. CC 173, 19°02'S, 163°18'E, bottom trawl, 250-290 m depth, 17 Sep. 1985, col. B. Séret; NSMT-P 54577, 1 spm 165 mm SL, same data as MNHN 1998-302; MUFS 14874, 1 spm 155 mm SL, same data as MNHN 1998-302; MUFS 14775, 1 spm dissected 137 mm SL, same data as MNHN 1998-302.

**Diagnosis**

A yellow sea bream of the genus *Dentex* characterized by: XIII + 9 dorsal fin rays; snout acute (70°-83°); eye diameter 2.40-3.35 times in head length; distal margins of dorsal and caudal fins bright yellow.

**Description**

Selected measurements and meristics are given in table I.

A yellow sea bream of the genus *Dentex* with an oval and compressed body; head large with anterior profile nearly evenly sloping, steeper in front of eye in large specimens; snout angle acute, 70° (holotype) and 70-83° (paratypes); snout and suborbital region naked; cheeks, opercle scaly; preopercle scaly except at its posterior margin; mouth low, slightly oblique; a single row of sharp, conical teeth along sides of each jaw, with 4 anterior canine-like teeth in upper jaw and 4-6 in lower jaw (character of *Denticinae*); interorbital slightly round and scaly; caudal fin forked.

Gillrakers on first arch 7 + 10 (holotype), 4-7 + 10-11 (paratypes); dorsal fin with 13 spines and 9 soft rays; the spines increasing in length up to the fourth or fifth, and subequal thereafter; anal fin with 3 spines and 8 soft rays; pelvic fin with 1 spine and 5 soft rays; pectoral fins with 15 soft rays (14 for 3 specimens); scales along lateral line 47 (holotype), 47-50 (paratypes); scales on transverse series 5 + 14 (holotype), 5-6 + 12-15 (paratypes).

Colour (in fresh specimens) (Fig. 2). - Body rosy to light red with silvery reflections; some specimens with purplish shades; dorsal and caudal fins pale yellowish with distal margin bright yellow; pectoral, pelvic and anal fins pale; base of pectoral fin pinkish; eye silver with some yellowish tint, iris black.

**Etymology**

The new species is named in honor of Pierre Fourmanoir, former ichthyologist in ORSTOM, who first collected this new sea bream in New Caledonian waters.

### Distribution

Known from Chesterfield Islands and New Caledonia from 210 to 290 m depth.

### COMPARISONS WITH OTHER SPECIES

(Figs 3, 4, Tables II, III)

The analysis of covariance performed on selected morphometric characters showed significant differences in some ratios (Table II).

Table I. - Selected measurements expressed as ratios of standard length (SL) and head length (HL) and meristic data of 3 Indo-Pacific species of *Dentex*.

Species	<i>Dentex fourmanoiri</i>	<i>Dentex tumifrons</i>	<i>Dentex sp.</i>
Locality	New Caledonia	Japan + China Seas	Okinawa + Ogasawara
Specimens examined	Holotype + 15 Paratypes	42 specimens	42 specimens
Standard length (mm)	226 (103-215)	68.5-247	205-316
RATIOS	Holotype (Range) Mean	Mean (Range)	Mean (Range)
SL / head length	2.94 (2.53-2.98) 2.77	2.82 (2.69-3.10)	2.94 (2.74-3.06)
SL / body depth	2.17 (2.04-2.26) 2.13	2.08 (1.97-2.88)	2.17 (2.00-2.36)
SL / body width	6.65 (5.15-7.63) 6.00	5.46 (5.20-6.68)	6.04 (4.63-7.50)
SL / pectoral fin length	2.69 (2.46-2.97) 2.70	2.66 (2.36-3.00)	2.72 (2.50-3.14)
HL / 1st dorsal spine length	5.13 (4.24-6.15) 5.02	4.40 (3.70-6.33)	4.69 (3.96-5.94)
HL / 2nd dorsal spine length	3.21 (2.83-4.13) 3.28	2.82 (2.45-3.62)	2.96 (2.43-4.05)
HL / 3rd dorsal spine length	2.37 (2.00-3.15) 2.43	2.18 (1.95-2.71)	2.26 (1.84-2.68)
HL / 1st anal spine length	4.28 (3.40-4.20) 3.74	4.35 (3.63-5.33)	4.05 (3.68-4.59)
HL / 2nd anal spine length	2.66 (1.89-2.59) 2.30	2.74 (2.37-3.20)	2.54 (2.26-3.10)
HL / 3rd anal spine length	3.35 (2.57-3.50) 2.93	3.00 (2.52-3.53)	3.02 (2.36-3.73)
HL / pelvic spine length	1.97 (1.80-2.38) 1.72	2.18 (1.93-2.47)	2.06 (1.83-2.36)
HL / pelvic fin ray length	1.51 (1.29-1.70) 1.47	1.46 (1.33-1.74)	1.43 (1.31-1.64)
HL / snout length	2.57 (2.12-2.65) 2.49	2.47 (1.85-2.97)	2.47 (2.26-3.21)
HL / upper jaw length	2.48 (2.25-2.93) 2.61	2.55 (2.32-2.78)	2.52 (2.28-2.79)
HL / interorbital width	3.21 (3.14-3.85) 3.46	3.44 (3.00-4.08)	3.19 (2.93-4.04)
HL / eye diameter	3.08 (2.40-3.35) 2.78	3.38 (2.84-4.40)	3.66 (3.09-4.09)
HL / suborbital width	4.05 (3.88-5.57) 4.62	3.91 (3.31-4.91)	4.12 (3.64-4.85)
MERISTICS	Holotype (Range) Mean	Mean (Range)	Mean (Range)
Lateral line scales	47 (47-50) 48.1	48.3 (46-50)	48.5 (47-50)
Transverse series: upper/under	5+14 (5-6+12-15) 5.5+13	5.4+14 (5-7+13-15)	5.8+14 (5-6+13-15)
Under 4th dorsal spine	5	5	5
Dorsal fin spines/rays	XIII+9	XII+10	XII+10
Anal fin spines/rays	III+8	III+8	III+8
Pelvic fin spines/rays	I +5	I+5	I+5
Pectoral fin rays	15	15	15
Gill rakers	7+10 (4-7+10-11)	7-8+11-13	7+11-12
Snout angle	70° (70°-83°) 76°9	82°9 (82°-88°)	80°8 (80°-83°)

Table II. - Main characteristics of the 10 known species of *Dentex*. DF = number of dorsal fin spines and rays; AF = number of anal fin spines and rays; PF = number of pectoral fin rays; SL = standard length; HL = head length; BD = body depth; SOW = suborbital width; LL = number of lateral line scales; TSL = number of transverse series of scales above the lateral line.

<i>Dentex</i> species	DF	AF	PF	SL/HL	SL/BD	HL/SOW	LL	TSL	Locality
<b>Atlantic</b>									
<i>D. angolensis</i>	XII-10	III-8	15-16	2.4-2.8	2.3-2.5	5.3-5.9	46-47	4	North-west African coast
<i>D. barnardi</i>	XII-10	III-8	16	2.9	2.5	4.1	61	6	West African coast
<i>D. canariensis</i>	XII-10	III-8	16	2.9-3.1	2.4-3.0	3.1-4.2	58-63	5-6	North-west African coast
<i>D. congoensis</i>	XII-10	III-8	15	2.9	2.6	7.2	46	4	North-west African coast
<i>D. dentex</i>	XI-11	III-8	15	2.8	2.6	4.0	60	8	England, France, Mediterranean, Morocco
<i>D. macrophthalmus</i>	XII-10	III-8	16	2.5-2.7	2.7-2.8	9.4-9.8	53-56	5-6	Portugal, Medit., Morocco, Mauritania
<i>D. maroccanus</i>	XII-10	III-8	16	2.7	2.3	5.2-6.5	48	4	North-west African coast
<b>Pacific</b>									
<i>D. tumifrons</i>	XII-10	III-8	15	2.7-3.1	2.0-2.3	3.5-4.7	46-50	5	Japan, East and South China Seas
<i>D. fourmanoiri</i>	XIII-9	III-8	15	2.6-3.0	2.1-2.2	4.1-5.6	45-50	5-6	New Caledonia
<i>Dentex</i> sp.	XII-10	III-8	15	2.7-3.1	2.0-2.3	3.6-4.9	47-50	5-6	Okinawa, Ogasawara

Table III. - Results of the covariance analysis on selected morphometric characters. HL = head length; BD = body depth; PL = pectoral fin length; 3DS = 3rd dorsal fin spine length; 3AS = 3rd anal fin spine length; SnL = snout length; SOW = suborbital width; ED = eye diameter; - = not significant. \* = significant at 5%; \*\* = significant at 1% level.

Covariance analysis	Covariance parameters	SL/HL	SL/BD	SL/PL	HL/3DS	HL/3AS	HL/SnL	HL/ED	HL/SOW
① Between <i>D. fourmanoiri</i> & <i>D. tumifrons</i>	Slope	0.2104	0.4303	0.983	4.5786	18.377	12.889	11.397	2.3829
	Adjusted mean	0.0118	5.1501	6.1451	0.1056	5.9339	6.6139	132.99	58.051
	Significant 5%	-	-	-	*	**	**	**	-
	Significant 1%	-	*	*	-	*	*	**	**
② Between <i>D. fourmanoiri</i> & <i>Dentex</i> sp. (Okinawa + Ogasawara)	Slope	0.0419	5.1064	0.0079	1.592	1.621	5.621	2.4258	0.0555
	Adjusted mean	4.9378	4.569	2.1396	7.5096	3.0111	0.5323	20.608	0.4457
	Significant 5%	-	*	-	-	-	*	-	-
	Significant 1%	*	*	-	**	-	-	**	-
③ Between <i>D. tumifrons</i> & <i>Dentex</i> sp. (Okinawa + Ogasawara)	Slope	0.0497	7.046	1.1454	0.1941	0.4946	0.448	0.4215	1.0448
	Adjusted mean	19.651	1.16	3.5297	1.8556	20.85	34.191	13.61	59.923
	Significant 5%	-	*	-	-	-	-	-	-
	Significant 1%	**	-	-	-	**	**	**	**

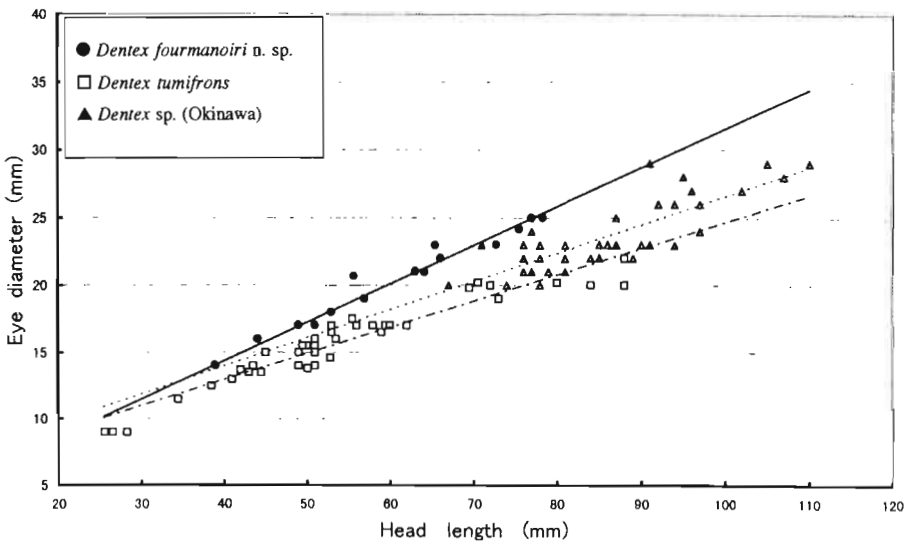


Fig. 3. - Regressions between head length and snout length in 3 Indo-Pacific species of *Dentex*.

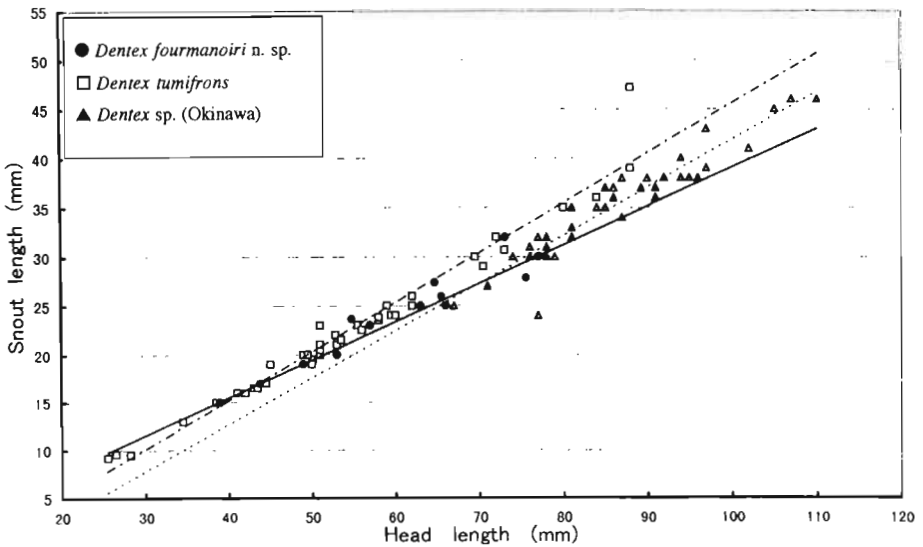


Fig. 4. - Regressions between head length and eye diameter for 3 Indo-Pacific species of *Dentex*.

*Dentex fourmanoiri* is mainly distinguishable from *Dentex tumifrons* by the number of dorsal fin rays (XIII + 9 versus XII + 10); snout angle more acute, 70°-83° versus about 78°-87°; a larger eye, its diameter 2.40-3.35 (2.78) times in head length versus 2.84-4.40 (mean: 3.38) (Fig. 3); general colouration light red with silvery reflections versus silvery red with golden sheen; dorsal and caudal fins with bright yellow margin versus fins orange-yellow to reddish; no blotches versus 3 large yellow blotches on body

below base of dorsal fin in *D. tumifrons*. Furthermore, *D. fourmanoiri* tends to have a shorter snout, its length contained 2.12-2.65 (mean: 2.49) times in head length versus 1.85-2.97 (mean: 2.47) (Fig. 4).

*Dentex fourmanoiri* is mainly distinguishable from an undescribed species from Japan, *Dentex* sp. (Akazaki in Masuda *et al.*, 1984) by number of dorsal fin rays (XIII + 9 versus XII + 10); a larger eye, its diameter 2.40-3.35 (mean: 2.78) times in head versus 3.09-4.09 (mean: 3.66) (Fig. 3); body without spots versus scattered with numerous small blue spots; dorsal fin with bright yellow margin versus dorsal bright yellow; pelvic and anal fins pale versus bright yellow in *Dentex* sp. Furthermore, *D. fourmanoiri* tends to have a snout angle somewhat more acute, 70°-83° versus about 80°-83°; a snout shorter, 2.12-2.65 (mean: 2.49) times in head versus 2.26-3.21 (mean: 2.47) (Fig. 4).

*Dentex fourmanoiri* is easily distinguishable from the 7 Atlantic species of *Dentex* by its number of dorsal fin rays: XIII + 9 versus XI-XII + 10-11 (Table III).

**Acknowledgements.** - Sincere appreciations go to the late Tokiharu Abe for offering specimens from the Ogasawara Islands, to Hiroshi Kohno for assistance in covariance analysis, to Jacques Rivaton, Bertrand Richer de Forges and René Grandperrin from the ORSTOM Centre in Noumea for collecting some of the specimens.

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## DEEP-SEA DEMERSAL ICHTHYOFAUNA OFF THE ST-PAUL AND AMSTERDAM ISLANDS (CENTRAL SOUTHERN INDIAN OCEAN)

by

Guy DUHAMEL (1)

**ABSTRACT.** - An oceanographic cruise (R.V. "Marion-Dufresne", July 1986) and an experimental fishing cruise (F.V. "Austral", July 1996) investigated the ichthyofauna of the deep-sea zone (350-3075 m and 400-980 m respectively) off the St-Paul and Amsterdam islands, a poorly known area in the central southern Indian Ocean. Forty-eight species belonging to 21 families were recorded. Macrouridae was the richest family in species, but in terms of abundance and biomass, Berycidae and Oreosomatidae seemed to be dominant in depths less than 1000 m. The ichthyofauna is compared to those from other areas of the temperate/subtropical belt in the southern hemisphere.

**RÉSUMÉ.** - L'ichtyofaune démersale profonde des îles St-Paul et Amsterdam (Océan Indien Sud).

Une campagne océanographique (N.O. "Marion-Dufresne", juillet 1986) et une campagne de pêche expérimentale (chalutier "Austral", juillet 1996) ont permis d'étudier l'ichtyofaune en zone profonde (respectivement 350-3075 m et 400-980 m) au large des îles St-Paul et Amsterdam, un secteur peu connu de la partie centrale sud de l'océan Indien. Quarante huit espèces appartenant à vingt et une familles ont été répertoriées. La famille des Macrouridae comporte le plus d'espèces, mais il semble qu'en terme d'abondance et de biomasse celles des Berycidae et des Oreosomatidae soient dominantes à des profondeurs inférieures à 1000 m. L'ichtyofaune est comparée à celles d'autres secteurs de la ceinture tempérée-subtropicale de l'hémisphère sud.

**Key-words.** - Deep-sea fish, ISW, St-Paul and Amsterdam Is., Inventory.

The volcanic St-Paul and Amsterdam islands are the only emergences of the south Indian mid-oceanic ridge branches. Their positions are located at mid-distance between the tips of southern Africa and Australia. The shallow-water ichthyofauna, first investigated by Kner (1865), has been recently revised (Duhamel, 1989). In terms of biogeographic affinities it belongs to the so-called "West Wind Drift Islands" province (Collette and Parin, 1991) which includes every island or seamount from the subtropical-temperate belt of the southern hemisphere. The mesopelagic ichthyofauna has also been investigated (Hulley, 1989; Hulley and Duhamel, 1990), but only preliminary data are reported (Duhamel, 1990) or summarized (Duhamel, 1997) for the deep-sea demersal fishes. Urgent knowledge of the deep-sea demersal ichthyofauna is needed because of the spreading of the deep-sea fisheries. Recent major surveys have conducted to advance in the ecological studies (Merrett and Marshall, 1981; Pearcy *et al.*, 1982; Gordon and Duncan, 1985, Merrett and Domanski, 1985) in Atlantic and Pacific Oceans but the central southern Indian

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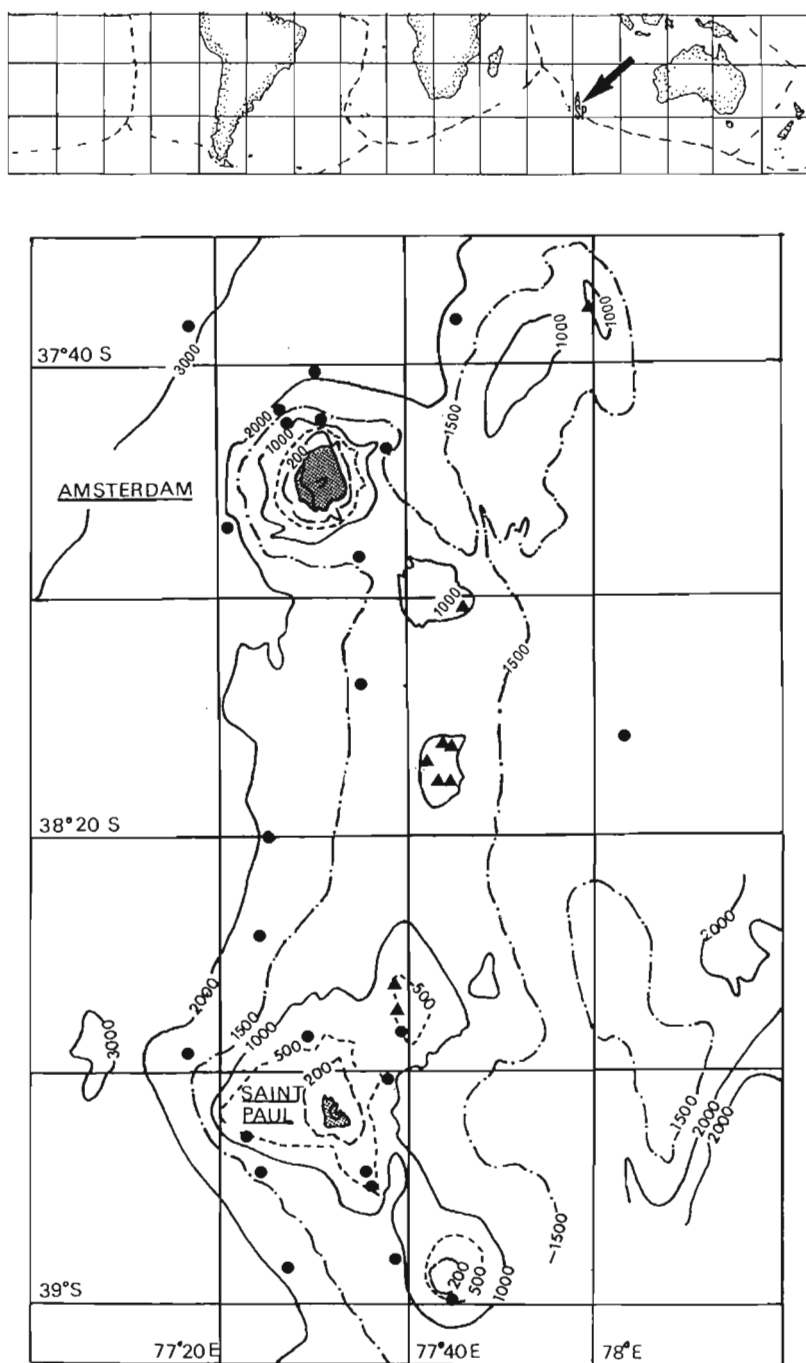


Fig. 1. - Position of the deep-sea scientific beam trawls (dots) and exploratory-fishing bottom trawls (triangles) conducted off the St-Paul and Amsterdam islands (SP and A at the tip of the arrow on the upper chart) during 1986 and 1996 respectively (N.B.: dashed lines on the upper chart indicate the mid-oceanic ridges).



Ocean still needs a first check-list. Recent trawling investigations allow advancement in the knowledge of this fauna.

## MATERIAL AND METHODS

The fish were collected during two cruises. The first was an oceanographic cruise (MD50/JASUS) of the R.V. "Marion-Dufresne" conducted during July 1986 (Arnaud, 1990). A series of 24 stations using a beam trawl (opening: one (height) x five (width) m; 10 mm mesh size in the codend) was carried out around the islands from the upper slope (350 m) to continental rise depths (3100 m) in a rectangled area ranging from 37°40'-39°00'S to 77°20'-78°00'E (Fig. 1). All the fish (104 specimens) collected were fixed on board, and later identified, registered, and stored in the MNHN collections. Some by-catch (five specimens) from dredges samples (three Charcot-Picard and one boulder dredges retrievals) from the same cruise and two catches of previous deep-sea baited-pot trials were also included in the study. The second cruise was an exploratory trawl survey of F.V. "Austral" conducted during July 1996 from 400 to 980 m on nearby seamounts in the same geographical area (Fig. 1). A commercial otter trawl (33.70 m of headline, 50 mm mesh size in the codend) was used for nine bottom trawlings (400-978 m). Information on the total catches was registered on a logbook. A fishery observer collected fish belonging to every species and labelled the specimens before storage in a deepfreezer. Catches were sometimes dominated by one to three species, in terms of number or weight, so sub-samples for collection purposes were necessary. One specimen of a shark (*Deania calcea*) was not retained, but photographs were taken for later identification. The bathypelagic species (n = 39) have been excluded from the present study, but the specimens were collected. Additionally benthopelagic fishes of one midwater trawl (75.4 x 59.4 m; vertical opening: 16 m) conducted close (six meters) to the bottom (824 m) were included in the analysis. The fish were fixed at the laboratory, identified, registered and lodged in the collection of the MNHN.

## RESULTS

A total of 48 demersal deep-sea species belonging to 21 families and 41 genera has been identified. The beam trawl catches, obtained from 18 positive hauls, and by-catch of pots and dredges include 30 species. The commercial bottom trawl (eight positive hauls) and the midwater trawl conducted close to the bottom contributed in the catches of 27 species. Details of the taxonomic identifications are given in table I. Some of these (*Caelorinchus*, *Etmopterus*, *Hydrolagus*, *Tubbia*) are still to the generic level because the need of further study. Damaged specimen (*Physiculus ?rhodopinnis*) does not allow to use fully a recent key (Paulin, 1989). The Macrouridae is the richest family in species (12 species) followed by Moridae (5 species). The other families are mainly represented by one or two species. The depth occurrence of the species from the results of the cruises (Table II) allows investigation of the depth distribution of the species.

Logbooks' data indicate that all the exploratory trawlings were stopped (winch alarm) before the end of the expected time because of rough bottom. Nevertheless, the relative catches were always dominated by two species: the spiky oreo, *Neocyttus rhomboidalis*, and the alfonsino, *Beryx splendens*.

Table I. - Identification of deep-sea species collected during the scientific (1986) and exploratory fishing (1996) cruises off St-Paul and Amsterdam islands (south Indian Ocean). The values indicate the number of collected specimens during the cruises. In case of partial sampling of the catch the number of collected specimens was expressed between brackets. \*: collected with pot.; \*\*: collected with mid-water net trawled close to the bottom.; \*\*\*: shallow-water species in their deeper bathymetric range.

Vessel Period Depth (m)	R.V. "MARION-DUFRESNE" July 1986 350 - 3100	F.V. "AUSTRAL" July 1996 400 - 978
Family	Species n	Species n
Squalidae	<i>Etmopterus granulosus</i> 5 <i>Etmopterus</i> sp. sensu Bass, Compagno & Heemstra, 1986 <i>Scymnodon macrocanthus</i> * 1	<i>Etmopterus granulosus</i> 1 <i>Deania calcea</i> 1 <i>Scymnodon macrocanthus</i> 1
Chimaeridae	<i>Hydrolagus</i> sp. sensu Compagno, 1986	
Synphobranchidae	<i>Diastobranchius capensis</i> 1 <i>Ilyophis blachei</i> 1	
Halosauridae	<i>Halosauropsis macrochir</i> 6	<i>Halosaurus pectoralis</i> 1
Moridae	<i>Antimora rostrata</i> 4 <i>Tripterophycis gilchristi</i> 3 <i>Lepidion capensis</i> 3	<i>Physiculus ? rhodopinnis</i> ** 1 <i>Tripterophycis gilchristi</i> 4 <i>Mora moro</i> ** 1
Macrouridae	<i>Caelorinchus karrerae</i> 7 <i>Caelorinchus</i> sp. sensu Iwamoto & Anderson, 1994 <i>Cetonus globiceps</i> 1 <i>Coryphaenoides mcmillani</i> 15 <i>C. serrulatus</i> 2 <i>C. carpinus</i> 1 <i>C. ? mediterraneus</i> 1 <i>Nezumia propinqua</i> 1 <i>Ventrifossa</i> cf. <i>ori</i> 2 <i>Spectrunculus grandis</i> 4	<i>Malacocephalus laevis</i> 4 <i>Caelorinchus</i> sp. sensu Iwamoto & Anderson, 1994 <i>Odontomacrus murrayi</i> ** 1 <i>Mesobius antipodum</i> ** 4
Ophidiidae		<i>Diplacanthopoma</i> cf. <i>nigripinnis</i> 1
Bythiidae		<i>Chaunax pictus</i> 2
Chaunacidae		<i>Beryx splendens</i> (8)
Berycidae		<i>Hoplostethus atlanticus</i> 3 <i>Hoplostethus mediterraneus</i> 4
Trachichthyidae	<i>Hoplostethus atlanticus</i> 2	<i>Dirtemoides parini</i> ** 1 <i>Cyttus traversi</i> (2) <i>Alloctytus verrucosus</i> ** 1 <i>Neocyttus rhomboidalis</i> (3) <i>Oreosoma atlanticum</i> ** 1 <i>Centriscoptus humerosus</i> *** 15
Dirtemidae		
Zeidae		
Oreosomatidae		
Macroramphosidae	<i>Centriscoptus humerosus</i> *** 1 <i>Notopogon xenosoma</i> *** 1	
Scorpaenidae	<i>Helicolenus mouchezi</i> *** 30 <i>Neomerinthe bauchotae</i> *** 4	<i>Helicolenus mouchezi</i> *** 5
Sebastolobinae	<i>Trachyscorpia capensis</i> 3	
Epigonidae	<i>Epigonus robustus</i> 1 <i>E. lenimen</i> 4	<i>Epigonus robustus</i> (2)
Uranoscopidae	<i>Pleuroscopus pseudodorsalis</i> * 1	<i>Pleuroscopus pseudodorsalis</i> 3
Stromateidae		<i>Tubbia</i> sp. 1 <i>Schedophilus maculatus</i> 1
Callionymidae	<i>Synchiropus phasis</i> *** 1	
Total deep-sea species / cruise	n = 30	n = 27
Total deep-sea species	n = 48	

## DISCUSSION

The present results give a still-incomplete list of the species occurring in the deep-sea environment off the islands, because the low number of successful trawls (26) in relation to the high number of damaged nets (five broken beams and failed nets; all bottom trawls stopped after less than ten minutes) reduced the positive catches. The topography of this area just on the top of ridge (Fig. 1) is the major cause of difficulty in sampling the deep-sea fish fauna with classical gear. In addition, comparison of cruise results is impossible due to the different selectivities and efficiencies of the two gear types used (mouth opening, mesh size, etc.).

Analysis of the catches on the upper-slope shows that some shallow-water species (*Centriscoops humerosus*, *Helicolenus mouchezi*, *Neomerinthe bauchotae*, *Synchiropus phasis*) are at the lower limit of their depth distribution. The deep-sea ichthyofauna appears at 400 m with the first species of Macrouridae and Moridae (*Ventrifossa* cf. *ori*, *Physiculus* ?*rhodopinnis*, *Tripteryophycis gilchristi*, *Caelorinchus* sp. *sensu* Iwamoto and Anderson, 1994). The most numerous species (33) are recorded from the upper slope off the islands or seamounts to about 1100 m. These species are also noted in other parts of the southern hemisphere in similar habitats such as off southern Africa (Smith and Heemstra, 1986; Bianchi *et al.*, 1993; Iwamoto and Anderson, 1994) (91% in common), southern Australia and New Zealand (Ayling and Cox, 1982; Last *et al.*, 1983; May and Maxwell, 1986; Amaoka *et al.*, 1990; Roberts, 1991; Gomon *et al.*, 1994; Williams *et al.*, 1996) (82% in common) but few (12%) in South America (Nakamura *et al.*, 1986; Pequeno, 1997). The typical families are Epigonidae, Berycidae, Oreosomatidae, Stromateidae, Trachichthyidae and Zeidae. Similarity with the thalassobathyal fish fauna of subtropical waters in the Indian Ocean is evident when the fauna is compared with those of the Madagascar Ridge/Walters Shoals, Mozambique Ridge, seamounts of the south-western and south-eastern Indian Ocean Ridge, Naturalist Plateau (Filatova, 1985; Shcherbachev, 1987; Pavlov and Andrianov, 1986; Parin *et al.*, 1993) (76% of the species in common), and Broken Ridge (P. Last, pers. comm.). A last group of nine species, recorded from about 1500 m and deeper, mainly belonging to families Macrouridae, Ophidiidae, Moridae and Halosauridae are worldwide in the bathyal depths. Haedrich and Merrett (1990) contend that no faunal zonation or communities occur in such deep-sea environments.

Noteworthy in the analysis of the catches is the apparent absence of two groups of fishes normally common in the deep-sea: the skates and the Alepocephalidae. The selectivity of the gear or the absence of suitable grounds for these near-bottom species could be an explanation for the absence of skates. However the hypothesis is not acceptable for Alepocephalidae which is abundant, for example, at the same latitude on the continental slope off western Australia (Williams *et al.*, 1996) and on the seamount slopes of the southern Indian Ocean (Shcherbachev, 1987). It is probable that the low number of trawls at appropriate depths (800-1200 m) of occurrence of the family is the reason for no representatives of the family having been taken.

The scientific beam trawl survey does not give the opportunity to know the dominance of species on the slope and at bathyal depths because of the scarce catches, which is related to the limited mouth opening of the gear. The survey of the seamount slopes is more interesting even if the low number of trawls or the short period of investigation (early winter) limit the conclusions. Three depth strata (400, 640-720, 980 m) were investigated. The first stratum provided mainly some specimens of the shallow-water ichthyofauna. Two species form the bulk of the catches in the second stratum: the spiky

Table II. - Depth range distribution of species collected during the scientific (1986) and exploratory fishing (1996) cruises off St-Paul and Amsterdam islands (southern Indian Ocean). Lighter greyish bars indicates the probable wider bathymetric range because trawling covered a very large depth interval.

Species	Depth (m)					
	500	1000	1500	2000	2500	3000
<i>Ventrifossa</i> cf. <i>ori</i>						
<i>Synchiropus phasis</i>						
<i>Notopogon xenosoma</i>						
<i>Neomerinthe bauchotae</i>						
<i>Physiculus</i> ? <i>rhodopinnis</i>						
<i>Caelorinchus</i> sp. sensu Iwamoto & Anderson, 1994						
<i>Centriscops humerosus</i>						
<i>Tripterygiopsis gilchristi</i>						
<i>Epigonus lenimen</i>						
<i>Helicolenus mouchezi</i>						
<i>Pleuroscopus pseudodorsalis</i>						
<i>Neocyttus rhomboidalis</i>						
<i>Beryx splendens</i>						
<i>Malacocephalus laevis</i>						
<i>Tubbia</i> sp.						
<i>Schedophilus maculatus</i>						
<i>Cyttus traversi</i>						
<i>Hoplostethus mediterraneus</i>						
<i>Oreosoma atlanticum</i>						
<i>Dirietmoides parini</i>						
<i>Scymnodon macrocanthus</i>						
<i>Halosaurus pectoralis</i>						
<i>Diplacanthopoma</i> cf. <i>nigripinnis</i>						
<i>Chaunax pictus</i>						

Table II. - (Continued).

Species	Depth (m)					
	500	1000	1500	2000	2500	3000
<i>Epigonus robustus</i>						
<i>Mesobius antipodum</i>						
<i>Mora moro</i>						
<i>Odontomacrus murrayi</i>						
<i>Deania calcea</i>						
<i>Etmopterus granulosus</i>						
<i>Lepidion capensis</i>						
<i>Hoplostethus atlanticus</i>						
<i>Coryphaenoides mcmillani</i>						
<i>Caelorinchus karrerae</i>						
<i>Diastobranchius capensis</i>						
<i>Trachyscorpia capensis</i>						
<i>Coryphaenoides serrulatus</i>						
<i>Nezumia propinqua</i>						
<i>Etmopterus</i> sp. sensu Bass, Compagno & Heemstra, 1986						
<i>Hydrolagus</i> sp. sensu Compagno, 1986						
<i>Ilyophis blachei</i>						
<i>Spectrunculus grandis</i>						
<i>Halosaurus macrochir</i>						
<i>Antimora rostrata</i>						
<i>Coryphaenoides carapinus</i>						
<i>Coryphaenoides ? mediterraneus</i>						
<i>Cetonus globiceps</i>						

oreo, *Neocyttus rhomboidalis*, and the alfonsino, *Beryx splendens*, with dominance of the first species at 650 m and the second at 715 m. Adult oreos are known to form large shoals over rough ground near pinnacles in Australia (Kailola *et al.*, 1993) a condition which seems presently to occur also off the St-Paul and Amsterdam islands. Alfonsino is a common targeted species on the seamounts of the nearby south-west branch of the mid-oceanic ridge (Fischer and Bianchi, 1984; Duhamel, pers. obs.) and its occurrence in this area is not surprising. An increase of the mean length with depth is clear for *Beryx splendens* (400 m: 25 to 35 cm in total length; 650-715 m: 25 to 60 cm,  $n = 450$ , mean = 38.1 cm; 980 m: few specimens from 57 to 60 cm) with diurnal migration in the open waters (results of the pelagic-trawl observations in the same area during the 1996 experimental cruise) as observed in other similar underwater rises (Vinnichenko, 1997). By-catch of large (15 to 35 cm) Epigonidae (*Epigonus robustus*) appears to be relatively common. Finally it appears that the orange roughy, *Hoplostethus atlanticus*, a species targeted by fisheries in southern Australia (Kailola *et al.*, 1993) and New-Zealand (Clark, 1996), is present in the deepest range of the bottom-trawl survey, but nothing about the abundance can be supported. Other species such as sharks (*Etmopterus*, *Deania*) and large-size *Beryx* occur also at these depths.

A more detailed survey of the area seems promising and could offer, in the near future, the opportunity to conduct an analysis of the deep-water fish assemblages from southern Africa to New Zealand similar to the studies of Merrett *et al.* (1991a, 1991b), Gordon *et al.* (1996) in the eastern North Atlantic, Stefanescu *et al.* (1993) in western Mediterranean, Newton and Klaer (1991), Koslow *et al.* (1994) in southern Australia and as initiated by Williams *et al.* (1996) in western Australia.

**Acknowledgements.** - The author wishes to thank the captains and the crew of the two vessels for the collection of the material. Special thanks are for the fishery observer, J.M. Le Petitcorps, who sampled the fish on board the "Austral". Help in the identifications from Dr. T. Iwamoto (for Macrouridae), B. Séret (for Squalidae) and O. Gon (for Epigonidae) is appreciated. The manuscript was improved by the useful comments of two anonymous referees.

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## THE SANDFISH, *GONORYNCHUS FORSTERI* (GONORYNCHIDAE), FROM BATHYAL DEPTHS OFF NEW CALEDONIA, WITH NOTES ON NEW ZEALAND SPECIMENS

by

Clive D. ROBERTS (1) & Terry C. GRANDE (2)

**ABSTRACT.** - The Australasian sandfish, *Gonorynchus forsteri* Ogilby, is recorded for the first time from the New Caledonian Exclusive Economic Zone (EEZ). The record is based on two adult specimens, one running ripe female and one spent or resting male, captured at 960-1233 m depth on the Loyalty Island Ridge and Lord Howe Rise. Their presence in bathyal depths over 700 nautical miles from the nearest known populations is discussed and compared with the occurrence of the species in New Zealand waters. It is hypothesized that adult sandfish migrate along oceanic ridges to spawn in southern New Caledonian waters.

**RÉSUMÉ.** - Le "sandfish", *Gonorynchus forsteri* (Gonorynchidae), des eaux profondes de Nouvelle-Calédonie et remarques sur des spécimens de Nouvelle-Zélande.

*Gonorynchus forsteri* Ogilby, d'Australasie, est signalé pour la première fois dans la Zone Économique Exclusive (ZEE) de Nouvelle-Calédonie. L'observation est basée sur la capture de deux spécimens adultes, une femelle prête à pondre et un mâle en repos sexuel, par 960-1233 m de profondeur sur les rides des îles Loyautés et Lord Howe. Leur présence à des profondeurs bathyales, à plus de 700 milles nautiques des populations les plus proches, est discutée et comparée à la distribution de l'espèce autour de la Nouvelle-Zélande. Nous émettons l'hypothèse que les "sandfish" adultes migrent le long de rides océaniques pour se reproduire dans la partie méridionale de la ZEE de Nouvelle-Calédonie.

**Key-words.** - Gonorynchidae, *Gonorynchus forsteri*, Sandfish, ISEW, New Caledonia, New record, Migration, Spawning.

Fishes in the genus *Gonorynchus* Scopoli, 1777 (family Gonorynchidae) are widely known in New Zealand as sandfish or sandeels because they live in coastal sandy habitats and have an elongate eel-like body. Sandfish are nocturnal, inhabiting shallow sandy bays and estuaries where they remain buried during the day (Graham, 1956; Ayling and Cox, 1982; Last *et al.*, 1983; Gomon, 1994; CDR, pers. obs.). However, they also occur in deeper water on the continental shelf (Ayling and Cox, 1982); for example, Last *et al.* (1983) recorded sandfish to 160 m depth off Tasmania, and Aizawa (1990) recorded sandfish at 104-678 m depth on the Chatham Rise and Challenger Plateau off New Zealand.

Sandfish in New Zealand waters, known variously as *G. gonorynchus* (L.), *G. greyi* (Richardson), or *G. forsteri* Ogilby, are recorded as common around both the North and

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South Islands, but are also known from the Kermadec Islands (Waite, 1910; Graham, 1956; Paulin and Stewart, 1985; Paulin *et al.*, 1989). Similarly, sandfish in eastern Australian waters, where they are known as *G. greyi* (Richardson), are recorded from coastal Tasmania to southern Queensland (Last *et al.*, 1983; Paxton and Hanley, 1989) and off-shore at Lord Howe Island (Ogilby, 1889) and Norfolk Island (Francis, 1991), but are more common in cool southern areas (Gomon, 1994).

Although quite a common fish locally in Australasian waters, there has been little work on the biology and life history of sandfish. They are thought to breed in deep water, and the young are transparent with a long pelagic postlarval stage (Last *et al.*, 1983; Smith, 1986). Off Japan, larval sandfish are epipelagic for several months, becoming benthic at 74–85 mm SL (Tsukamoto and Okiyama, 1993). Off New Zealand, juvenile sandfish are pelagic until a similar size (and presumably also age). Thus, a long pelagic stage in the life cycle allows wide dispersal of juveniles.

The type species of the genus, *Gonorynchus gonorynchus* (Linnaeus, 1766), originally described from the Cape of Good Hope, has been recorded widely in the cooler parts of the Indo-Pacific region (Nelson, 1984), including New Zealand (Waite, 1907) and Chile in the South Pacific and St. Helena in the South Atlantic (Smith, 1986).

Nominal species of *Gonorynchus* include: *G. abbreviatus* (Temminck & Schlegel, 1846) from southern Japan; *G. moseleyi* Jordan & Snyder, 1923, from Hawaii; *G. greyi* (Richardson, 1845) from southwest Australia; *G. parvimanus* Ogilby, 1911 from eastern Australia; and *G. forsteri* Ogilby, 1911 from New Zealand (Fowler, 1941; Whitley, 1968; Last *et al.*, 1983; Aizawa, 1990; Gomon, 1994). The original descriptions of these species are problematic, and the nominal species are poorly distinguished, being separated primarily on geographic location, overlapping morphometric characters and variable colour differences which change during development. The genus is currently being revised by the second author (Grande, in press).

*Gonorynchus* (type and only living genus of the family Gonorynchidae) is a basal ostariophysan, and one of the few exclusively marine groups within the predominantly freshwater superorder. Sandfishes share with other gonorynchids (i.e., † *Notogoneus* from Eocene freshwater deposits of North America, Europe and Australia, and † *Charitosomus* from Cretaceous marine deposits of the Middle East and Germany) the presence of conical teeth on the endopterygoids and basibranchial 2, as well as a distinctive caudal skeletal morphology (Grande, 1996). A minimum age of the Late Cretaceous for the Gonorynchidae indicates that the group is ancient, predating the breakup of Gondwana (Smith and Briden, 1977). According to Fink and Fink (1981, 1996) and Grande (1994, 1996) the Gonorynchidae is most closely related to a small group of African freshwater fishes (Knerioidei) with no known fossil record.

*Gonorynchus* exhibits many distinctive morphological specializations which are most likely habitat adaptations. The two newly discovered bathyal specimens described herein clearly belong to *Gonorynchus* in having the following diagnostic characters: long cylindrical body, conical head with a protrusible ventral mouth, large eye (about 20% of head length) covered by transparent tissue, median sensory barbel on the ventral side of the snout, a sensory barbel within the mouth extending from the roof, epibranchial organ present, long fleshy pectoral and pelvic axils, posterior placement of the dorsal, pelvic and anal fins, a lateral line that extends past the hypural plate ending on the caudal fin rays, absence of a swimbladder (Fowler, 1936; Nelson, 1984; Grande, 1996), and strong peripheral ctenoid scales (Roberts, 1993).

The present paper reports the occurrence of sandfish for the first time from remote bathyal depths in the southwest Pacific Ocean, thereby being a new record for the New Caledonian fish fauna. Voucher specimens, collected during the French exploratory cruise "HALIPRO 2" and held in the Muséum national d'histoire naturelle, Paris (MNHN), verify this record. These important specimens are described and compared with conspecifics in the collection of the Museum of New Zealand (NMNZ) (acronyms used for institutions follow the international standards set by Leviton *et al.*, 1985), and we suggest reasons why *Gonorynchus forsteri* is present on the Loyalty Island Ridge and Lord Howe Rise.

### *GONORYNCHUS FORSTERI* OGILBY, 1911

(Pl. 1, Table 1)

#### Material examined

New Caledonia: MNHN 1997-727, 463 mm SL, female, R.V. "Tangaroa", cruise "HALIPRO 2", station BT 28, seamount "Loyalty South" (25°23.65'S, 170°29.86'E to 25°24.47'S, 170°30.46'E), Loyalty Island Ridge, 960-1011 m depth, sea surface temperature 22.2°C, bottom temperature 5.9°C, orange roughy bottom trawl, 11 Nov. 1996, 10:35-10:52 h, bottom time 17 min, distance trawled 0.98 nautical miles. MNHN 1997-728, 389 mm SL, male, R.V. "Tangaroa", cruise "HALIPRO 2", station BT 95, Lord Howe "flat" (23°56.86'S, 162°7.57'E to 23°59.80'S, 162°08.21' E), Lord Howe Rise, 1224 m depth, sea surface temperature 24.2°C, bottom temperature 4.2°C, orange roughy bottom trawl, 25 Nov. 1996, 13:10-14:11 h, bottom time 61 min, distance trawled 3.0 nautical miles.

New Zealand: 63 specimens, 65-545 mm SL (Appendix 1 and NMNZ unpubl.).

#### Diagnosis

The New Caledonian *Gonorynchus* specimens described in this paper are identified as *G. forsteri*, which differs from all other nominal *Gonorynchus* species in the total number of vertebrae, number of lateral line scales, and the pattern of lateral line scales. *G. forsteri* is defined by having counts of total vertebrae of 64-66, caudal vertebrae (= preural caudal vertebrae + ural caudal vertebrae) 12 + 1, total lateral line scales 240-256, and lateral line scale pattern of 2:2:2 (i.e., two scales: pore, two scales: pore, etc.) (Grande, in press). Meristic and measurement data for the two New Caledonian specimens are summarized in table I.

Diagnostic vertebral counts (taken from radiographs) of 64 ( $n = 22$  specimens), 65 (34) and 66 (7) confirm that most New Zealand sandfish specimens held in NMNZ are *G. forsteri*, the same species found off New Caledonia. A few specimens from northern New Zealand, including those from the Kermadec Islands, belong to another species with 60 vertebrae, probably *G. greyi* which is found predominantly off Australia (Grande, in press).

#### Colour pattern

*Fresh specimens.* - Female, 463 mm SL (Pl. 1A): body countershaded, brown dorsally and pale pink ventrally; pupil blue-black, iris golden with dusky flecks; fins dark grey, without marked white or black blotches, base of paired fins orange-brown.

Male, 389 mm SL (Pl. 1B): body sandy-brown dorsally, pale salmon-pink ventrally but not strongly countershaded; fins dark grey, caudal fin with two large dusky

blotches. New Zealand male, 430 mm SL, (Pl. 1C): body countershaded, brown dorsally and salmon-pink ventrally; pupil blue-black; fins orange-brown basally with dark grey bands distally, caudal with pale angles.

*Preserved specimens.* - Female: marked contrast in pigmentation between the dorsal 2/3 of the fish, which is very dark, almost black, and the ventral 1/3 which is a light cream colour; fins heavily pigmented; base of paired fins orange/rust colour.

Male: specimen darker over all, dorsal 2/3 darker than ventral 1/3, but not as sharp a contrast as seen in the other specimen; fins heavily pigmented. New Zealand male: countershaded, dorsal 2/3 brown, ventral 1/3 orange-tan; fins orange-tan basally, black distally; dorsal fin with pale posterior angle, pectoral with pale margin, pelvic and anal with pale anterior margin and angle, caudal with pale median patch and angles.

### Comments on coloration

Fresh sandfish have a distinctly countershaded body colour pattern, comprising brown dorsally and orange-pink ventrally, and fins with dark grey-black blotches contrasting with pale patches and margins. This coloration is largely retained in preservative, except for the ventral body colour which becomes pale tan. The two New Caledonian specimens exhibit this basic colour pattern, but its intensity is reduced or masked by dark pigment when compared to New Zealand specimens (e.g., Pl. 1). For example, the female had dark grey fins without marked black or white blotches, and the male was not strongly countershaded (Pl. 1A, B). Possibly the intensity of colour pattern and amount of dark masking pigment present are influenced by depth of habitat.

Table 1. - Diagnostic meristic and morphometric data from two New Caledonian specimens of the sandfish *Gonorynchus forsteri* Ogilby. (\*: Several posterior lateral line scales missing).

	MNHN 1997-727 « HALIPRO 2 » Stn BT 28 463 mm SL	MNHN 1997-728 « HALIPRO 2 » Stn BT 95 389 mm SL
<b>Counts</b>		
Dorsal fin rays	10	10
Anal fin rays	7	7
Pectoral fin rays	10	10
Pelvic fin rays	9	9
Total vertebrae	65	65
Total caudal vertebrae	12 + 1	12 + 1
Total lateral line scales	248*	256
<b>% Standard length</b>		
Predorsal length	70.3	69.4
Pectoral to caudal peduncle	78.8	79.7
Pelvic to caudal peduncle	32.2	32.6
Anal to caudal peduncle	8.9	9.7
Head length	18.7	19.7
<b>% Head length</b>		
Eye length	19.0	21.0

### Comments on capture

The two New Caledonian specimens were collected in a standard New Zealand orange roughly bottom trawl which has the following characteristics: door spread 120 m, distance between wing tips 75-80 m (but lower panel absent); headline length 38 m, ground rope length 22 m, wing spread 25 m, mouth width 10 m, headline height above ground line 5.5-7.5 m, cod end mesh 10 cm with (for bycatch research) liner and cover with 4 cm mesh (stretched).

Although it is sometimes possible for fishes to be collected by bottom trawls during their descent and ascent from the bottom, this is unlikely in the present case. When setting this trawl there is little forward movement of the net; the vessel steams ahead mainly to unwind the trawl warps. Without forward movement the trawl cannot fish effectively. Once on the bottom, the trawl is towed at a speed of approximately 3 knots.

During cruise "HALIPRO 2", the tow at station BT 28 was stopped after a period of 17 min and a bottom distance of under 1 nautical mile because the trawl became stuck on the rough bottom. Before it was freed from the bottom snag the net had become tangled and twisted and could not have fished on the way up (Malcolm Clark, pers. comm.). Therefore, it is reasonably certain that the first specimen of sandfish was in fact captured on the bottom at a depth of 960-1011 m. In addition to fishes, the net sampled sponges and corals, indicating it had successfully trawled the seabed.

The tow at station BT 95 was over flat hard ground for just over 1 hour without mishap. As discussed above, it is unlikely that the second sandfish specimen could have been caught during the descent of the net to the bottom. It could have been collected in mid-water during the 20 min it took to haul the net to the surface. Nevertheless, based on the duration of the tow, it seems most likely that the 405 mm SL specimen was captured on the bottom at 1224-1233 m depth. Although no benthic invertebrates were captured, the presence of benthic fishes, including a ray, an ogocephalid and some synphobranchid eels, indicated that the net was successfully fishing on the bottom.

After a short period of sorting on deck, the specimens were rapidly cooled in iced water and photographed to record fresh coloration (Pl. 1A, B), before being fixed in 10% formalin and preserved in 70% ethanol. Observations on the sandfishes made immediately after capture showed the eyes to be clear and the gills to be bright red; conditions not seen in old, frozen or preserved specimens. Therefore, there can be no doubt about the authenticity of these fish as fresh specimens. They could not, for example, have been brought into the area, accidentally or otherwise, on board the New Zealand R.V. "Tangaroa".

### Comment on maturity

The first and larger New Caledonian specimen was running ripe when picked up on deck. Examination of its gonads showed the fish to be a ripe female with large ovaries full of approximately 0.5 mm diameter white-coloured eggs (note swollen abdomen of specimen in Pl. 1A). Many eggs were free in the ovary lumen and could be extruded from the oviduct with gentle pressure on the abdomen. In addition, it was evident when handling this specimen that the body muscles were flaccid and watery, being almost transparent when viewed through an incision in the skin.

The second specimen was in better physical condition, the body being turgid and the muscles solid and opaque gray-white in colour. It was a male in either a spent or resting state. If it had been spawning, there was no indication from gross gonad appearance or somatic state that it had happened recently.

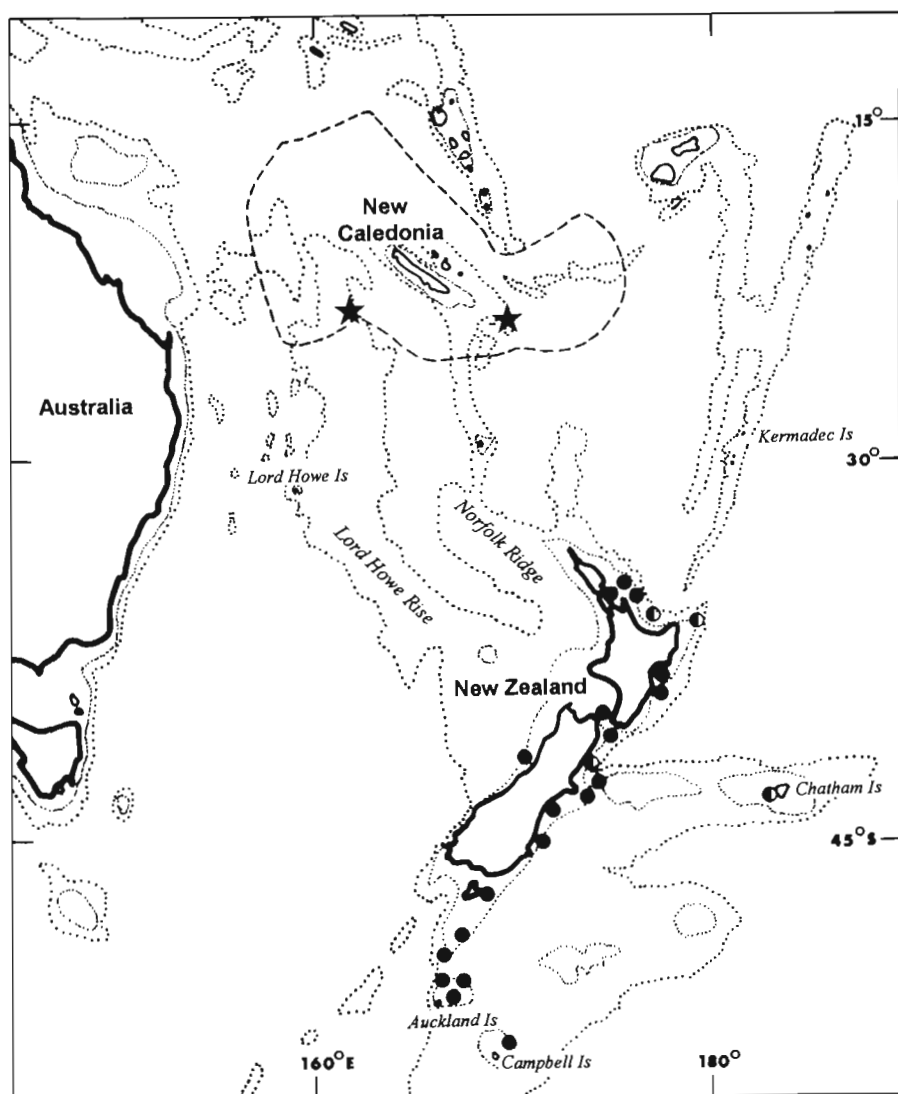


Fig. 1. - The southwest Pacific region showing capture locations of the sandfish, *Gonorynchus forsteri* Ogilby: two specimens caught during cruise "HALIPRO 2" held in MNHN Paris (★); and specimens of benthic adults (●) and pelagic juveniles (●) held in NMNZ Wellington. Dashed line shows limit of New Caledonian EEZ; bathymetry shown as 500 and 2000 m contours.

#### Comments on New Zealand *Gonorynchus forsteri*

In New Zealand waters, juveniles, 65-90 mm SL, have been captured by dip net at the surface at night and in fine mesh trawls near the surface over deep water, off White Island, East Cape, Kaikoura, and Chatham Island (Fig. 1). Benthic juveniles, 92-220 mm SL, have been taken at 1-37 m depth by dredge, hand net and spear in east coast harbours, estuaries, bays and off open beaches, between Auckland and Foveaux Strait.

Subadults and adults, 280-545 mm SL, taken mostly by trawl, are generally more abundant off southern coasts and have been collected off Great Barrier Island in the north to as far south as the subantarctic Auckland and Campbell Island plateaus (Fig. 1). The deepest-caught specimen (NMNZ P.30722, 512 mm SL) was trawled at 613-625 m depth off Auckland Island; 24 other adults in 20 lots have been captured at depths between 20 and 470 m (mean depth 243 m,  $n = 21$  lots) (Appendix 1).

Thus, whilst juvenile, subadult and adult *G. forsteri* do occur in shallow coastal areas, as cited in the literature, adults also inhabit deep shelf and slope areas and isolated offshore plateaus, and are presumably capable of moving between the two. However, none of the New Zealand specimens were taken from habitats approaching the extreme depths of the two New Caledonian specimens.

#### Comments on distribution of *Gonorynchus forsteri*

The present records of *G. forsteri* thus show a remarkable distribution, extending from 23°57'S to 52°12'S, a distance of approximately 1,700 nautical miles (3,150 km), crossing three broad climate zones (subtropical, temperate, and subantarctic), and two biogeographic/hydrographic barriers (the tropical convergence and subtropical convergence).

The two New Caledonian specimens were caught over 700 nautical miles away from the nearest known resident population off New Zealand.

### DISCUSSION

*Gonorynchus* species have not previously been recorded from New Caledonian coastal waters (Rivaton *et al.*, 1989) or from the adjacent waters of the Great Barrier Reef and Coral Sea (Randall *et al.*, 1990). The presence of sandfish in remote oceanic trawls made at depths of 960 to 1233 m was, therefore, most surprising since the fish is familiar to ichthyologists in New Zealand and Australasia where it is generally thought to be a relatively shallow-living, coastal species.

It is probably significant that the first specimen collected was a running ripe female clearly in the act of spawning. The second specimen was a male, which although not ripe, may have spawned sometime previously. Therefore, it is hypothesized that these two captures form part of the tail end of an adult spawning migration from coastal Australasian waters along oceanic ridges into southern New Caledonian waters (Fig. 1).

The long postlarval pelagic phase of sandfish would enable wide dispersal from a deep spawning ground off New Caledonia. Recruitment of postlarval sandfish into coastal New Zealand waters is theoretically possible because the tropical convergence zone south of New Caledonia is weak or absent during the summer period (Stanton, 1969) and warm subtropical water moves towards New Zealand, particularly during La Niña periods of the Southern Oscillation cycle (Francis and Evans, 1993).

Evidence in support of long distance recruitment of sandfish is (1) the apparent absence of larvae or small-sized postlarvae in New Zealand waters, (2) the capture of 16 large pelagic postlarvae, 65-92 mm SL, in offshore New Zealand waters during summer months (Fig. 1; NMNZ, unpubl. data), and (3) the observation that in some years large numbers of benthic juvenile sandfish, 15 cm in length, occur seasonally during the spring and summer months (Graham, 1956) suggesting periodic settlement of annual cohorts.

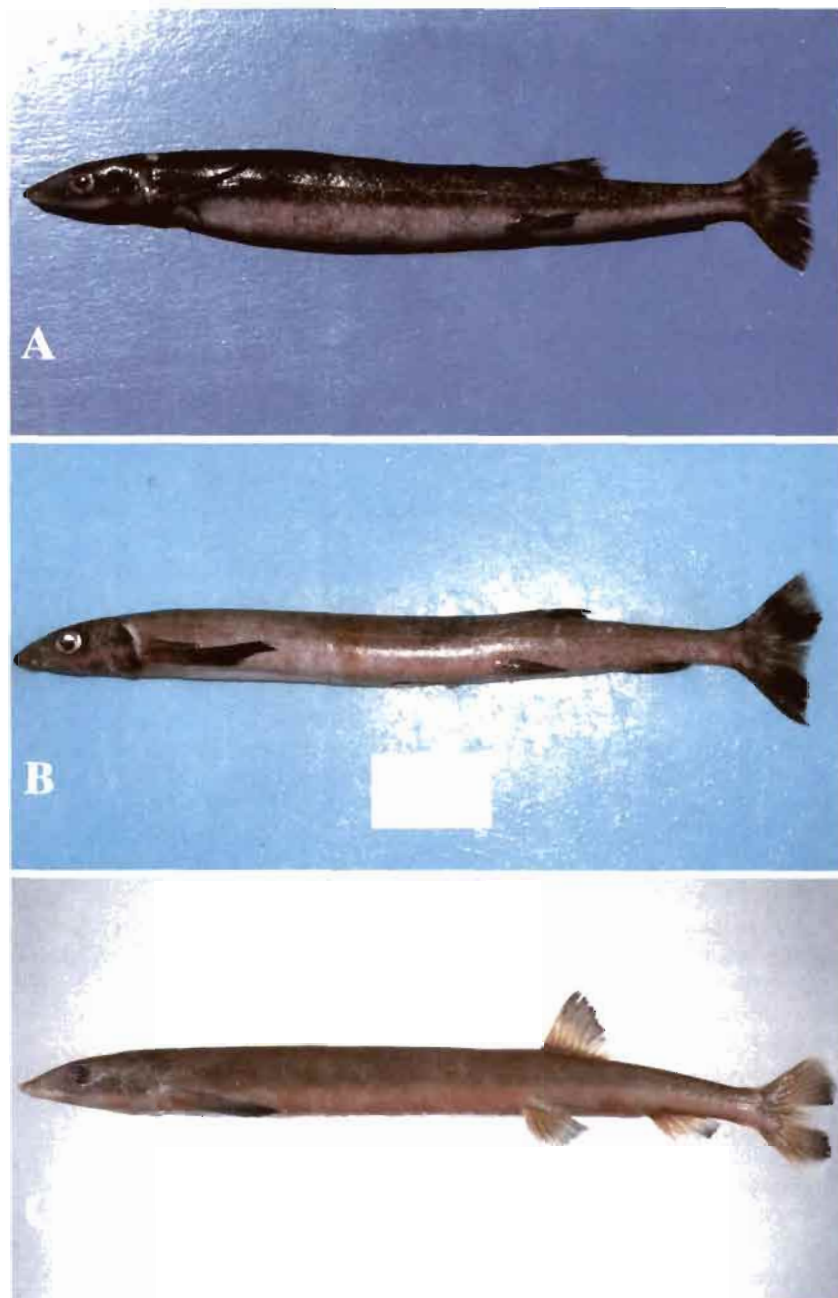


Plate 1. - Fresh sandfish, *Gonorynchus forsteri* Ogilby. **A:** New Caledonian specimen 463 mm SL, female (MNHN 1997-727), 960-1011 m depth, Loyalty Island Ridge (photo P. Laboute, ORSTOM Nouméa). **B:** New Caledonian specimen 389 mm SL, male (MNHN 1997-728), 1224-1233 m depth, Lord Howe Rise (photo C. Roberts, MNZTPT). **C:** New Zealand specimen 430 mm SL, male (NMNZ P.33703), 51 m depth, Canterbury Bight (photo A. Stewart, MNZTPT).



Corroboration of this hypothesis of a spawning migration of sandfish will require the capture of *G. forsteri* larvae or spawning sandfish during future fish surveys in southern New Caledonian waters, particularly during the months of September and October. To this end, it is notable that on the evening of 26 October 1991 a large aggregation of unidentified fishes on the bottom at 1200-1500 m depth was observed on the echo sounder by the first author on the western side of the Loyalty Island Ridge during ORSTOM research cruise "BERYX 2" (Grandperrin and Lehodey, 1992).

The reason for a spawning migration from Australasian waters into distant northern bathyal depths is not obvious. However, the reason may be historical rather than ecological. Sandfish belong to a very old group of fishes and the bathymetric features of the present northern Tasman Sea formed coastal and shelf areas of continental Gondwana some 70 M years ago. Possibly sandfish today are following a similar spawning route to that of their Gondwanan ancestors.

**Acknowledgements.** - We thank the directorates of the French ZoNéCo programme and ORSTOM Centre de Nouméa for inviting the first author to participate in exploratory cruise "HALIPRO 2". Thanks to René Grandperrin and Bernard Séret (ORSTOM), and Malcolm Clark, Dianne Tracey, Brent Wood, Captain Roger Goodison, officers and crew of the New Zealand R.V "Tangaroa" (NIWA) for their help during the "HALIPRO 2" survey. We thank Bernard Séret, Antenne ORSTOM, Muséum national d'histoire naturelle, Paris, for the loan of preserved specimens; Mark Westneat and Kevin Swagel, Field Museum of Natural History, Chicago, for the use of the Fish Division's x-ray machine and dark room facilities; and Robin McPhee and Andrew Stewart, Museum of New Zealand, Wellington, for preparing radiographs and providing technical assistance. The manuscript was critically reviewed by Peter Castle, Victoria University of Wellington, and Tomio Iwamoto, California Academy of Sciences, San Francisco. Work on NMNZ specimens was part funded by New Zealand Foundation for Research, Science and Technology contract MNZ603 Biosystematics of NZ EEZ Fishes Project.

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Appendix 1. - Location and depth of adult specimens of the sandfish *Gonorynchus forsteri* Ogilby, held in the Museum of New Zealand (NMNZ) fish collection. Listed by increasing size.

NMNZ Reg. N°	SL mm	Location	Position		Depth m	Date
			Lat. S	Long. E		
P.7406	385	E. Chatham Rise	43°18.10'	174°55.50'	220	11 Dec. 1977
P.31740	386	off Cape Kidnappers	39°37.31'	177°45.14'	204	17 Jan. 1995
P.24206	400	N. Campbell Plateau	48°49.10'	166°47.50'	159-165	29 Jan. 1989
P.34302-2	400-453	off Banks Peninsula	44°10.13'	173°33.26'	320-321	08 Jan. 1997
P.34300	414	off Banks Peninsula	43°47.57'	174°04.34'	387-391	16 Dec. 1996
P.5193-2	415-450	off Hokitika	43°22.00'	169°22.00'	315-348	03 Nov. 1970
P.31671	423	E. of Auckland Island	50°48.00'	166°47.00'	400-410	01 Oct. 1994
P.33703	430	Canterbury Bight	44°05.81'	172°22.42'	51	07 Jun. 1996
P.24207	430	Auckland Is. Rise	50°14.00'	166°26.20'	162-167	26 Jan. 1989
P.34303-3	436-493	off Timaru	44°40.84'	172°26.57'	205-211	09 Jan. 1997
P.33397	443	E. of Auckland Is.	51°00.60'	166°29.60'	181	23 Feb. 1996
P.1145	445	Wellington Hbr.	41°14.00'	174°50.00'	20	07 Sep. 1952
P.31064	460	Campbell Plateau	48°48.70'	166°42.90'	200-234	19 Feb. 1991
P.24891	465	E. of Auckland Is.	50°03.50'	167°50.40'	260-279	23 Oct. 1989
P.26796	480	N. Campbell Plateau	49°58.00'	166°22.00'	180	29 Mar. 1991
P.5341	510	Cook Strait	40°59.00'	173°06.00'	24-28	10 Mar. 1971
P.33034	510	E. Chatham Rise	43°10.00'	175°44.00'	430-470	Oct. 1995
P.30722	512	N. of Auckland Is.	49°30.31'	166°12.70'	613-625	11 May 1993
P.33020	515	Foveaux Strait	46°55.00'	168°15.00'	53	02 Oct. 1995
P.26814	545	Auckland Island Rise	51°00.20'	166°30.30'	180	28 Jan. 1988



## Symposium 5

### CHONDRICHTHYAN FISHES

Chairperson: **Bernard SÉRET**

- HOMMA K., MARUYAMA T., ITOH T., ISHIHARA H. & S. UCHIDA. - 209-216  
 Biology of the manta ray, *Manta birostris* Walbaum, in the Indo-Pacific.  
*Biologie de la raie manta, Manta birostris Walbaum, dans l'Indo-Pacifique.*
- BONFIL R. - Marine protected areas as a shark fisheries management tool. 217-230  
*Utilisation des zones marines protégées comme outil de gestion des pêcheries de requins.*
- CARVALHO M.R. de - A synopsis of the deep-sea genus *Benthobatis* Alcock, 231-255  
 with a redescription of the type species *Benthobatis moresbyi* Alcock, 1898 (Chondrichthyes, Torpediniformes, Narcinidae).  
*Révision du genre Benthobatis Alcock, et nouvelle description de son espèce-type Benthobatis moresbyi Alcock, 1898 (Chondrichthyes, Torpediniformes, Narcinidae).*
- FOWLER S.L., MANJAJI M.B., CAVANAGH R.D., COMPAGNO L.J.V., MY-COCK S.G. & P.R. LAST. - Elasmobranch biodiversity, conservation and management in Sabah (Malaysia). 257-269  
*Biodiversité, conservation et gestion des élasmobranches au Sabah (Malaisie).*
- HAMLETT W.C., HYSELL M.K., JEZIOR M., ROZYCKI T., BRUNETTE N. & K. TUMILTY. - Fundamental zonation in elasmobranch oviducal glands. 271-280  
*Zonation fondamentale des glandes nidamentaires chez les élasmobranches.*

- HAMLETT W.C., HYSSELL M.K., ROZYCKI T., BRUNETTE N., TUMILTY K., HENDERSON A. & J. DUNNE. - Sperm aggregation and spermatocyte formation in the male genital ducts in the clearnose skate, *Raja eglanteria*. 281-291  
*Agrégation du sperme et formation de spermatocytes dans les conduits génitaux mâles de la raie à nez blanc, Raja eglanteria.*
- LAST P.R. & B. SÉRET. - Comparative biogeography of the chondrichthyan faunas of the tropical south-east Indian and south-west Pacific Oceans. 293-306  
*Biogéographie comparée des faunes chondrichthyennes tropicales de l'Océan Indien tropical du sud-est et de l'Océan Pacifique du sud-ouest.*
- NAKAYA K. & K. SATO. - Species grouping within the genus *Apristurus* (Elasmobranchii: Scyliorhinidae). 307-320  
*Groupements d'espèces dans le genre Apristurus (Elasmobranchii, Scyliorhinidae).*
- TANIUCHI T. & H. TACHIKAWA. - Geographical variation in age and growth of *Squalus mitsukurii* (Elasmobranchii: Squalidae) in the North Pacific. 321-328  
*Variations géographiques de l'âge et de la croissance de Squalus mitsukurii (Elasmobranchii: Squalidae) dans le Pacifique Nord.*
- TESHIMA K., TODA M., KAMEI Y., UCHIDA S. & M. TAMAKI. - Reproductive mode of the tawny nurse shark, *Nebrius ferrugineus* (Elasmobranchii: Ginglymostomatidae) in Okinawa waters, with comments on individuals lacking the second dorsal fin. 329-333  
*Mode de reproduction du requin-nourrice fauve, Nebrius ferrugineus (Elasmobranchii: Ginglymostomatidae) dans les eaux d'Okinawa, et remarques sur des individus dépourvus de seconde nageoire dorsale.*
- YANO K., YABUMOTO Y., TANAKA S., TSUKADA O. & M. FURUTA. - Capture of a mature female megamouth shark, *Megachasma pelagios*, from Mie, Japan. 335-349  
*Capture d'une femelle mature de requin grande gueule, Megachasma pelagios, au large de Mie, Japon.*
- YANO K. - Chondrichthyans of the Ryukyu Islands, Japan. 351-365  
*Chondrichthyens des îles Ryukyu, Japon.*

## BIOLOGY OF THE MANTA RAY, *MANTA BIROSTRIS* WALBAUM, IN THE INDO-PACIFIC

by

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Hajime ISHIHARA (3) & Senzo UCHIDA (4)

**ABSTRACT.** - The systematics, biology, captive biology, color variations, fisheries, and conservation of the manta rays are reviewed based on the literature and field observations of authors and other sources. Only one species, *Manta birostris*, may exist in the genus *Manta*, in contrast to nine species in the genus *Mobula*. Based on the identification using the variations in ventral color patterns and traces of shark bite, biology of the rays have been clarified. Also, the observation of the rays in captivity in the Okinawa Expo Aquarium provided significant information concerning their biology. Black rays appear to be a color variant of the manta ray and are distributed widely in the whole Pacific Ocean, except for the Yap, Palau and Hawaii Islands. Populations of manta rays due to heavy fishing on both coasts of the Pacific Ocean have collapsed in Mexico and are now threatened in Philippines. To prevent collapse, it is necessary to start the campaign of ecotourism for manta rays in developed countries in Asia.

**RÉSUMÉ.** - Biologie de la raie manta, *Manta birostris* Walbaum, dans l'Indo-Pacifique.

La révision de la systématique, la biologie, la biologie en captivité, les variations de couleur, les pêcheries et la conservation des raies manta sont abordées en tenant compte de la littérature ainsi que des observations de terrain par les auteurs et d'autres sources. Il existe apparemment une seule espèce dans le genre *Manta* contre neuf pour le genre *Mobula*. La biologie des raies a pu être clarifiée grâce à l'identification des individus en utilisant leur coloration ventrale et les traces des morsures de requins. La biologie en captivité a pu être étudiée par l'observation des raies de l'Okinawa Expo Aquarium. Les raies noires semblent être une forme de raie manta et leur distribution s'étend sur tout l'Océan Pacifique sauf pour les îles Yap, Palau et Hawaii. Les populations de raie manta ont chuté considérablement au Mexique et sont menacées aux Philippines. Il est nécessaire d'envisager des campagnes d'écotourisme dans les pays développés en Asie afin d'éviter une chute irréversible des effectifs.

**Key-words.** - Myliobatidae, *Manta birostris*, Black manta, Indo-Pacific, Systematics, Biology, Captive biology, Fisheries, Conservation.

The manta ray, *Manta birostris* is a circumtropical, large mobulid ray, considered to be monospecific in the genus *Manta*, in contrast to nine species in the genus *Mobula*. The former genus is distinctive from the latter by the terminal location of mouth and absence of teeth in the upper jaw. The manta attains a size of at least 6.7 m in disc width (DW) and a maximum size of 9.1 m DW (Last and Stevens, 1994). Although the ray is

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famous for its size, wide distribution, and peaceful behaviour, almost nothing has been reported for its ecology except for small descriptions of the species by Michael (1993) and Ishihara and Homma (1995). The population of the ray has collapsed or is threatened in both coasts of the Pacific Ocean. We describe the systematics, biology, fisheries, and conservation of the ray.

## SYSTEMATICS

Recently Nishida (1990) included the genera *Manta* and *Mobula* in the family Myliobatidae, but Nelson (1994) retained the subfamily Mobulinae in the family Myliobatidae with two genera *Mobula* Rafinesque and *Manta* Bancroft. The genus *Mobula* is distinct from the genus *Manta* by the ventral location of mouth and presence of teeth on both jaws. The systematics of the genus *Mobula* was well studied by Notarbartolo-di-Sciala (1987) who considered nine species to be valid. The genus *Ceratobatis* Boulenger, which was described as lacking teeth on lower jaw, was synonymized with the genus *Mobula* by Cappetta (1987). Also the genus *Indomanta* Whitley, whose mouth is located terminal with teeth on both jaws, was considered to be congeneric with the genus *Mobula* by Bigelow and Schroeder (1953).

Four species of the genus *Manta* have been described, i.e., *Manta birostris* (Donndorff, 1798), *M. americana* (Bancroft, 1828), *M. hamiltoni* (Newman, 1849) and *M. alfredi* (Krefft, 1868), but we agree with the opinion of Eschmeyer *et al.* (1983) who regarded that there should be only one world-wide species, *M. birostris*. Randall *et al.* (1997) considers Walbaum (1792) to have the priority with regard to the species name *birostris*.

## BIOLOGY

All the following information was obtained by Takashi Itoh, who has been living in the Yaeyama Islands, based on the identification method unless otherwise stated.

### Identification method

Concerning the biology of the species, identification is useful because every individual of the species have unique features such as patterns of dark markings on the belly and evidence of shark bites. Takashi Itoh has been successful in the identification of each manta ray based on these features. He has identified about 185 individual manta rays near the Yonara channel between the Iriomote and Obama Islands in the past twenty years until summer of 1997. The same method has been employed by Bill Acker, who lives in the Yap Island and operates the Manta Ray Hotel in the island. He has been successful in identifying 54 individual manta rays near the island in the past seven years up to autumn 1997. Staffs of the Kona Surf Resort (Manta ray village) on the Hawaii Island have also used the same method, to identify the manta rays that migrate to shore at night because planktonic crustacean swarms in the night illumination of the hotels. Other identification methods has been employed by Charles Anderson (Ministry of Fisheries and Agriculture, Male) in the Maldives Islands. He started the tagging survey for the manta rays of the area in February 1996.



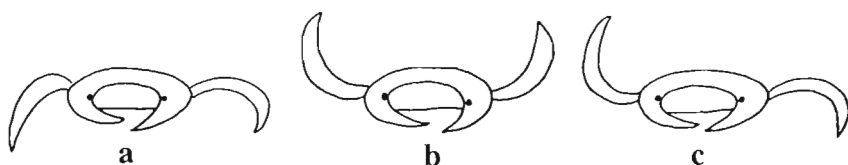


Fig. 1. - Three types of swimming style in the manta ray. a: omega-shaped; b: U-shaped; c: S-shaped.

Takashi Itoh named each manta ray due to its features, i.e. "Peace" (= V-shaped dark mark), "Sakana" (= Fish-shaped mark) and "Mitsuboshi" (= tri-star mark), etc. and he has taken photo of each of them. Bill Acker has also named each manta ray and taken photos of them for the record.

### Migration

During the day, manta rays migrate from feeding stations to cleaning stations. At their feeding stations, manta rays swim slowly at the surface layer and are occupied in feeding. At their cleaning stations, manta rays hover just above the bottom layer and wait to be cleaned by a wrasse (*Labroides dimidiatus*) or small shrimps. While many manta rays stay near the Yaeyama Islands all year round, some migrate annually to another island such as Kerama Island, which is about 350 km east of the Yaeyama Islands.

In the Yap Island, manta rays are confined to the island and do not leave, which has been ascertained by Bill Acker based on his 12 years of observations (pers. comm, B. Acker, 1997). In the Maldives Island, Charles Anderson also noted that a manta ray tagged at the end of one monsoon was resighted by a diver at the beginning of the same monsoon the following year. He considered that the ray migrate from one plankton-rich side of the islands to other and back again in phase with the seasonally changing monsoons (pers. comm., C. Anderson, 1997).

According to Alava and Trono (in press), the Philippines fishermen set gill nets at almost the same channel in order to catch manta rays through fishing season. The diving instructors of Maldives Islands and Caroline Islands usually lead the divers who want to watch manta rays to the same diving spot each time. Therefore, the daily migratory pass of the manta rays seems to be somewhat regular.

### Schooling

Schooling was commonly observed by Takashi Itoh, but the school size has reduced from 50 in number (17 years ago) and 30 in number (seven years ago) to at most 14 to 15 in number presently. It, however, does not mean that the total number of rays in the area have decreased. Young rays and pregnant females also make up the same school, but no other fish including other mobulids are involved.

### Swimming style and jumping behaviour

S-shaped, U-shaped and omega-shaped swimming styles have been recorded (Fig. 1). Jumping behaviour is often observed. It appears that the rays do this for fun, and that this behaviour does not seem to relate to parturition or the removal of parasites and remoras as Clark (1969) suspected. In order to remove remoras, they press their belly against the rocks. Three types of jumping are observed: jumping forwards and landing head first, jumping forwards and landing tail first, and backward somersaults.

### **Mating behaviour**

In the Yaeyama Islands, copulation has not been seen although mating behaviour are often observed, i.e., male and female rays somersaulting vertically together in the water column. This behaviour is observed in spring and autumn and lasts for as long as one month. Since manta rays do not have teeth on the upper jaw and the teeth on lower jaw are non-functional, it is believed that male manta ray is not capable of biting the pectoral of females doing copulation.

### **Reproduction**

One female called "Mitsuboshi" (= Tri-Star) was seen to bear a single pup three times during a six to seven year period. Therefore, the pregnancy may last up to two or three years. Parturition was not observed, although this has been recorded in the magazine *Skin Diving* in a volume of about 1975 to 1977. Nursing behaviour was not evident, the new-born pup was left alone in the water after birth. Age at maturity may be about six years. Females with disc width from 4.3 m to 4.6 m contained an embryo in the uterus and a male with disc width 3.5 m was immature (Bigelow and Schroeder, 1953).

### **Age and size**

One male ray, which appeared to be some four to five years old when first sighted, has been observed over 15 years. Therefore, it is considered that the rays life span is more than 19-20 years. Individuals which attain the disc width of 4 m are commonly seen. Maximum disc width of 6.7 m and weight of 1,361 kg was reported from the Bahama Islands (Bigelow and Schroeder, 1953) and is said to attain disc width of 7.3 m with more than 2 metric tons (Misra, 1969). In Japan, the largest individuals recorded were one from Wakayama whose disc width was about 5 m (Yanagisawa, 1994) and one female collected from Okinawa waters whose disc width was 4.65 m and 774 kg in weight (Uchida, 1994). It seems that the female attains a larger size than the male. The minimum free-swimming juvenile was 1.22 m DW and the maximum embryo was reported to be 1.27 m DW and 9.1 kg in weight and 1.14 m DW and 12.7 kg weight (Bigelow and Schroeder, 1953). Therefore, the size of the manta ray at parturition might be from 1.1 m to 1.3 m DW.

### **Predator and parasite**

Sharks may be the most common predator of manta rays, because marks from shark bites are often seen. However, it is uncertain whether sharks eat the whole body of manta rays. Killer whales are believed to be another predator of manta rays, but they are not seen near the Yaeyama Island. In some manta rays, the traces of shark bites, which seem to be caused by the cookie-cutter shark, were observed. Therefore, it is suspected that manta rays often swim downwards to more than 100 m depth.

Though whitish parasites are sometimes observed on the dorsal side of manta rays, they were not collected and thus not identified. It seems that this parasite is a pandarid copepod similar to parasite that attaches to *Mobula lucasana* (Benz and Deets, 1986). Remoras are regarded as a famous parasite to devil rays, but mantas sometimes try to remove them away by pressing their body to the rocks or sandy bottom.

### **Distribution in Japan**

The northernmost record of the manta ray in Japan is from the Izu Peninsula (35°N) on the Pacific coast and from Hagi, Yamaguchi prefecture (34.5°N) on the coast of the Sea



Fig. 2. - Black manta ray in the Yaeyama Islands. Photo by Takashi Itoh.

of Japan (unpubl. data and Uchida, 1994). Misra (1969) reported that the manta rays are distributed in the waters where average water temperature would not decrease below 20°C.

## MANTA RAYS IN CAPTIVITY

### Okinawa Expo Aquarium

The Okinawa Expo Aquarium, the director of which is Senzo Uchida, was built in 1975 in the memory of the Okinawa Ocean Expo, and located in Motobu, Okinawa Island. The aquarium is the only aquarium in the world that keeps manta rays. At present three manta rays are living there in captivity. The first one is a female collected near Ie, Okinawa on the 3rd November, 1988 and was 2.3 m DW when collected. The second one is a male collected near Kanna, Okinawa on 25th May, 1992 and was 1.75 m DW when collected. The third one is a male collected near Kanna on 5th May, 1993 and 2.1 m DW when collected. The first one has been kept in the aquarium for nine years and the second and third ones for five years and a half and four years and a half respectively.

### Feeding

Foods for those manta rays are euphausiids, *Euphausia pacifica* and *E. superba* and sakura shrimp, *Sergestes lucens*. The weight of food consumed per week is 12.7% of the total weight (Uchida, 1994). The foods of manta rays in nature are reported to be small shrimps, crabs and small fishes (Bigelow and Schroeder, 1953).

### **Color variant - Black manta ray**

Manta rays whose ventral surface is dark are popularly called black mantas. Faint white markings on the dorsal side are absent in the black manta, which makes them distinguished from a dorsal view. In the Pohnpei Island, about 30% of manta rays are "black" (unpubl. field obs., Homma and Ishihara). The black mantas were first reported from the Pacific coast of Panama by Barton (1948) and have since been reported from Yaeyama Islands (unpubl. field obs., T. Itoh, Fig. 2), Sulu Sea (pers. comm., Michiyo Nishitani, Ten knot Tours, 1995), Exmouth Gulf (pers. comm., Tony Medcraft, Whale shark Research Center, 1997), New Caledonia (pers. comm., Claire Garrigue, ORSTOM, 1997), Tahiti (pers. comm., Takeshi Iwato, South Sea Pearl, 1995), Hawaii Islands (pers. comm., Tim Clark, Texas A&M Univ., 1998) and Socorro Island (pers. comm., Akira Tateishi, Marine Planning, 1995). They have not yet been reported from the Yap, and the Palau Islands (pers. comm., B. Acker, Katsutoshi Itoh, 1995-1997) despite being observed over most of the entire Pacific Ocean. In the Maldives Archipelago in the Indian Ocean, the black manta has not yet been found (pers. comm., C. Anderson, 1993).

Since the black manta and normal manta are sympatric and no other morphological characters was found except for coloration, they may belong to the same species. At Pohnpei island, the black manta and normal manta rays were observed coupling together (unpubl. field obs., Homma and Ishihara).

## **FISHERIES**

### **Fishing activity**

Fisheries for manta rays exist on both coasts of the Pacific Ocean, i.e., off Mexico and the channels between islands of middle and southern Philippines. Also, relatively large number of manta rays are taken in gillnet fisheries in Sri Lanka and India. Notarbartolo-di-Sciara (1988) reported that fishing activity for mobulids exist in the village of the southernmost part of the Gulf of California. Fisheries for whale shark and mobulids in Philippines were reported by Alava and Trono (in press). According to their report, fisheries exist in the Bohol Sea (Sulu Sea) near Visayas Islands and Mindanao Island. Using drift nets (700 to 1,000 m long and 35 m height) and harpoons, fishermen of only one island community (Pamilacan Island) caught more than about one thousand mobulids including manta rays in only one fishing season (actually from December, 1995 to next May, 1996). The drift gill nets were set at the time of migratory passages of the mobulids. As many as 50 manta rays were usually collected in a single gill net about 7 m below water surface. The size of the manta rays taken ranged from 1.5 m to 6 m DW.

### **Price**

Price of one manta ray with 4 m DW was \$155 and 6 m DW \$310, respectively. After the meat is dried, it is sold at a price of \$7 to \$10 per kg. The liver was salted, divided into six pieces and sold for \$7.75 per 5 gallon container. The gill rakers were also dried and sold for \$7.75 per kg. Both liver and gill rakers are believed to be good for health and processed into Chinese traditional medicine.

In 1997, an additional and more extensive survey was conducted by WWF, Philippines and Silliman University, in which the manta ray and whale sharks catches were obtained from 12 or more landing sites in the Pamilacan Island, Bohol; Catarman and Sagay, Camiguin; and Talisayan, Misamis Oriental (Alava and Trono, in press).

## CONSERVATION

### **Manta rays now threatened in the Sulu Sea**

Manta rays are easy to catch, because of their large size, slow swimming speed and the lack of fear of humans. The collapse of the mobulid fishery in Mexico was already reported by Notarbartolo-di-Sciara (1995). Populations of manta rays and whale sharks in Bohol Sea are also threatened (Alava and Trono, in press). An estimated 12,000 mobulids including manta rays are killed by fishermen every year (based on one thousand ray at each of 12 landing sites). A Japanese amateur diver, Ms. Michiyo Nishitani (Ten knot tours) who dives in the Palawan Island has suggested that the local population of manta rays has fallen to one half to one third in the past seven years.

### **Ecotourism**

A dead manta ray is worth \$400, so the annual maximum income from fisheries will be near \$4,800,000 in Philippines. We suggest the divers of developed countries encouraged to watch manta rays in the Philippines. If each diver spends \$400 per person in those islands, it means that the island may invite only 12,000 tourists to the islands instead of catching manta rays.

We would like to propose a campaign to invite about 12,000 Japanese divers to the Philippines who could pay money directly to the community of those islands. In this way, the population of rays is maintained while still benefiting the local economy.

**Acknowledgements.** - Sincere thanks are extended to the following persons who provided us with important information concerning the manta rays: Bill Acker, Charles Anderson, Claire Garrigue, Katsutoshi Itoh, Takeshi Iwato, Issei Katayama and the staffs of Phoenix, Pohnpei, Mike McCoy, Tony Medcraft, Michiyo Nishitani, Akira Tateishi. Romy Trono kindly sent us the Ms. for the manta rays fishery in Philippines.

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## MARINE PROTECTED AREAS AS A SHARK FISHERIES MANAGEMENT TOOL

by

Ramón BONFIL (1)

**ABSTRACT.** - Marine protected areas (MPAs) can be classified into three broad categories according to their main objectives: ecological (conservation of biodiversity and habitats), economic (fisheries enhancement and protection), and social (tourism, education). In practice, many MPAs fulfill several or all of these objectives at the same time. The utilisation of no-take marine reserves as a fisheries management tool has been applied to a variety of resources, most commonly teleosts from tropical and temperate waters. However, there is very limited experience in the usage of MPAs for the protection or enhancement of shark stocks. This paper presents a review of worldwide information on the protection status of sharks. The results indicate the existence of only one no-take marine reserve used as a fisheries management tool for sharks. However, there are several *de facto* MPAs for sharks that have different objectives and which offer various degrees of protection. General criteria commonly utilised for the design of MPAs are reviewed while attempting to evaluate their application and feasibility for the implementation of MPAs for different kinds of sharks.

**RÉSUMÉ.** - Utilisation des zones marines protégées comme outil de gestion des pêcheries de requins.

Les zones marines protégées (MPAs) peuvent être classées en trois grandes catégories selon leur objectifs: écologiques (conservation de la biodiversité et des habitats), économiques (accroissement des pêcheries et de la protection), et sociales (tourisme, éducation). En pratique, la plupart des MPAs ont plusieurs, sinon tous ces objectifs simultanément. L'utilisation des réserves marines totales (sans aucun prélèvement) comme outil de gestion des pêcheries, a été appliquée à des ressources diverses, mais le plus communément aux téléostéens des mers tropicales et tempérées. Il y a très peu d'expérience d'utilisation des MPAs pour la protection et la reconstitution des populations de requins. Le présent article est une revue mondiale de l'information disponible sur la protection des requins. Il montre qu'il n'existe qu'une seule réserve marine totale utilisée comme outil de gestion pour les populations de requins. Cependant, il existe *de facto* pour les requins plusieurs MPAs qui ont d'autres objectifs et qui offrent des degrés variables de protection. Les caractéristiques principales utilisées pour l'établissement de MPAs sont passées en revue, et un essai d'évaluation de leur application et de la faisabilité de leur mise en oeuvre pour différentes espèces de requins est présenté.

**Key-words.** - Sharks, Marine protected areas, Reserves, Fishery management.

Marine Protected Areas or MPAs can be classified broadly according to the objectives they try to accomplish. Ecological objectives can be: conservation of biodiversity, habitats or ecosystems, and the protection of endangered species. Examples of social objectives are recreation, education and research. There can be also economic objectives such as fisheries management, or protection of human settlements (as in the case of tourist facilities that depend on coral reefs for protection of the sandy beaches they rely on).

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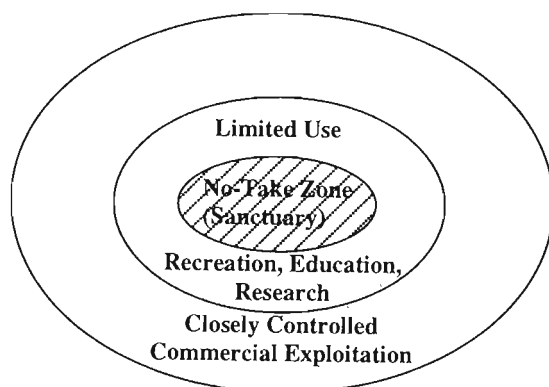


Fig. 1. - Zoning allows the achievement of multiple objectives in MPAs. (Modified from Salm and Clark, 1984).

When a zoning approach is chosen, MPAs can fulfil several objectives at the same time (Fig. 1).

Marine protected areas can offer different levels of protection for species or ecosystems. There are MPAs that allow limited harvesting of living resources, others that do not allow any kind of harvest, and some that go even further by limiting other types of human activity such as recreational uses (diving, boating, etc.).

To be effective for fisheries management, no-take MPAs are supposed to perform two jobs: to provide refugia to prevent or relieve stock collapse, and to enhance and help maintain fishery yields in nearby areas. Note that in order to perform these jobs, MPAs must be able to sustain viable populations.

Table I lists the range of advantages and disadvantages offered by long-term MPAs. This list includes some points of especial relevance to shark populations. Among the advantages, MPAs can provide undisturbed spawning/breeding grounds, and sharks, having internal fertilisation and being mostly live-bearers will probably benefit substantially by having a safe place to mate and give birth to their extremely vulnerable juveniles. Marine protected areas also offer potential economic benefits through increased tourism. With the emergence of shark diving as a growing popular activity, economic benefits will become more and more relevant for shark protection. On the down side, MPAs are not considered effective for highly migratory species. Also, to be effective they are supposed to include all types of habitat occupied ontogenetically by the species. As discussed below, these two aspects render MPAs difficult to apply to many sharks.

In this paper I will first introduce the current state of shark protection through the different types of MPAs, then I will outline the general principles for the design of no-take MPAs. This will be followed by an evaluation of how these principles fit shark life history characteristics using some hypothetical examples. Finally, I will summarise the usefulness and limits of MPAs as fisheries management tools for elasmobranchs.

### **Global status of protection for sharks**

Existing MPAs for sharks can be classified into 3 categories: a) virtual or *de facto* MPAs, where governmental legislation has designated certain species as protected in waters within their jurisdiction; b) official MPAs for sharks, which are areas designated



specifically for protection of shark species and which are usually oriented towards ecological goals; and c) no-take MPAs intended as a fisheries management tool for sharks.

By far, the most common are the first kind, the virtual MPAs which occur in several parts of the world. Official MPAs for sharks occur only in two places, Florida and the Maldives, and no-take MPAs for fisheries management only exist in Australia.

### Protected shark species or "informal MPAs" for sharks

There are at least five elasmobranch species protected through specific legislation (informal MPAs) around the world (Table II). Due to its high profile and its vulnerability to overexploitation, the white shark (*Carcharodon carcharias*) is the most commonly protected shark species around the world. White sharks are protected in South Africa, in California state waters, in all federal waters bordering the USA coasts of the Gulf of Mexico and the Atlantic Ocean (5.5-370.5 km from shore except Texas and the West coast of Florida where federal waters are those between 18.5-370.5 km from shore), and in most of Australia (Tasmania, New South Wales, Queensland, South Australia, Western Australia, and all Commonwealth waters) with the exception of the state waters of Victoria. The whale shark (*Rhincodon typus*), is protected in Florida state waters and all federal waters of the USA coasts of the Gulf of Mexico and the Atlantic Ocean, in the Maldives, and in Western Australia. However, it is questionable whether this provides sufficient protection for whale shark populations because migratory routes are poorly known. The basking shark (*Cetorhinus maximus*) is protected in waters of Florida, all federal waters of the USA

Table I. - Advantages and disadvantages of long-term Marine Protected Areas. (Modified from Plan Development Team, 1990, after Russ *et al.* 1994). Italics denote points of special relevance for elasmobranchs.

Advantages	Disadvantages
Lower chance of recruitment overfishing (critical spawning stock)	Concentration of fishing effort on smaller portion of stock
Increased or maintained yield in broad areas (larval dispersion)	Possible reduction of short term yields due to smaller available stock
Increased or maintained yield in adjacent areas by adult fluxes	Benefits might become evident only in the long-term
Undisturbed spawning/breeding ground	Incentive for poaching
Maintenance of interspecific and intraspecific genetic diversity	Increased need for intensive surveillance
Unfished populations for scientific research	Strong local resistance in proposed areas
Reduced management costs (minimal information needed)	Uncertainty about size, location and number of reserves to ensure persistence of the species
Economic benefits through tourism	Long-term detailed research needed to justify spatial closures
Concept easy to understand and accept by general public	Not useful for highly migratory species
Simplified enforcement	Resistance of managers to "new approaches"
Reduced temptation to violate laws by fishermen	Should ideally include habitat for all life stages
Habitat protection	
Areas for education use	
Provides source of broodstock for possible restocking of depleted areas	

coasts of the Gulf of Mexico and the Atlantic Ocean, the Isle of Man, the Mediterranean Sea, and New Zealand. The sandtiger shark (*Carcharias taurus*), is protected in New South Wales, Queensland, and all Australian Commonwealth waters, and also in all federal waters of the USA coasts of the Gulf of Mexico and the Atlantic Ocean. The big eye sandtiger shark (*Odontaspis noronhai*) is protected in all federal waters of the USA coasts of the Gulf of Mexico and the Atlantic Ocean. Other elasmobranchs protected in Florida state waters are the sawshark (*Pristiophorus schroederi*), the spotted eagle ray (*Aetobatus narinari*), and sawfish (*Pristis* spp.). Israel currently protects all chondrichthyan species but the level of protection is unknown (no commercial or sport fisheries?). Finally, the Barcelona Convention includes protection for several species in the Mediterranean Sea, the white shark, the basking shark and the giant devil ray (*Mobula mobular*), which are considered endangered or threatened. However, the levels of protection are unknown.

### Formal MPAs for sharks

There are only two examples of formal MPAs specifically designated for the protection of sharks. These are the MPA for grey reef sharks (*Carcharhinus amblyrhynchos*) in the 15 top diving sites of the Maldives, and the MPA of the Dry Tortugas, Florida, for the protection of the nurse shark (*Ginglymostoma cirratum*) during its mating season from May to August (Carrier and Pratt, 1996).

### No-take MPAs as fisheries management tools for sharks

The only known no-take marine reserves for sharks used as fisheries management tools are in Australia. The first, is an area closed to shark fishing that extends over a significant portion of the coast of Western Australia (Shark Bay to North-West Cape). This closed area is controlled through gear restrictions (no gillnets and no lines with metal traces can be used). This does not eliminate the taking of sharks, but catches in the region are minimal. Most of the catch occurs in trawls and consists of smaller bottom-dwelling species. This MPA is designed to protect the breeding stock of larger species such as dusky (*Carcharhinus obscurus*) and sandbar sharks (*C. plumbeus*) that form the basis of the fishery in the southern part of the state. The second case are some selected inshore areas around Tasmania and Victoria which are closed to the southern shark fishery in order to protect breeding aggregations of school shark (*Galeorhinus galeus*) and gummy sharks (*Mustelus antarcticus*).

Table II. - Shark and ray species protected by informal MPAs (legislation protecting species in jurisdictional waters).

Species	Location of informal MPAs
White shark	South Africa, USA (California state waters, federal waters of Gulf of Mexico and Atlantic coast), Australia (Tasmania, New South Wales, Queensland, South Australia, Western Australia, and all Commonwealth waters from 5.5 km to edge of EEZ)
Sandtiger shark	Australia (NSW for 12 years, Queensland, and all Commonwealth waters from 5.5 km to edge of EEZ), USA (all federal waters of Gulf of Mexico and Atlantic coast)
Big eye sandtiger shark	USA federal waters of Gulf of Mexico and Atlantic coast
Whale shark	Australia (Western Australia); Maldives; USA (Florida state Waters (6.6 km Gulf, 5.5 km Atlantic), all federal waters of Gulf of Mexico and Atlantic coast)
Basking shark	Isle of Man, Manx territorial waters (22.2 km); USA (Florida state Waters, all federal waters of Gulf of Mexico and Atlantic coast)
Sawfish and sawshark	Florida state waters
Spotted eagle ray	Florida state waters

### The design of no-take MPAs

As a general principle, to be effective as stand-alone fisheries management tools, long-term MPAs must ideally include a good mix of suitable habitats that provide protection to all the life stages of the species in question. In other words, they must be able to support viable populations. This is perhaps their most important characteristic and also the most difficult to meet.

Geographic size is another important criterion in the design of marine reserves. Because MPAs should be sufficiently large to support a viable population, in order to select the appropriate size it becomes necessary to have information on home ranges, rates of movement in and out of the reserve, migrations, etc. According to Ballantine (1991), a rule of thumb for defining the size of an MPA is a few km<sup>2</sup> as minimum, while the maximum should be defined by practicality and spacing of replicate sites. The geometry of the reserve is usually defined using easily identifiable special features to delimit the area.

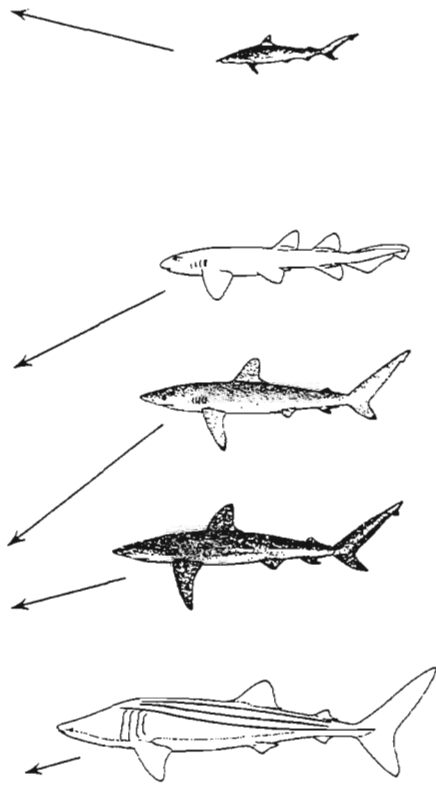
Site selection must be within the distribution range of the species. An important consideration is that generally, habitat suitability is higher towards the centre of distribution of the species (MacCall, 1990). Also, the proximity of the site to other important areas such as fishing ports, heavily populated human settlements, or other reserves, needs to be considered. It should be also acknowledged that the social acceptance of no take MPAs will vary from country to country according to the cultural environment and the traditions of natural resource ownership.

A complex problem for the design of natural reserves is the SLOSS question. This problem arises from the debate over whether it is better to have, a Single Large Or Several Small reserves? This point is not easy to resolve and there are different view points among experts. Systems or networks of smaller MPAs seem to be favoured by some authors who stress some advantages such as the provision of more robust systems and simplified design (Ballantine, 1991). Large marine reserves are proposed by others who argue that some species could not survive if large areas are not set aside for their protection (i.e., some hard corals; Salm and Clark, 1984). On the other hand, it has been suggested that small interconnected MPAs of different habitats forming functional large MPAs might be a viable solution to local resistance to form large MPAs in heavily populated areas.

### Sharks and the design of MPAs

The first point to consider is that there is no such thing as "the" shark, the "average" shark or the "typical" shark. The diversity of the 400 or so species of sharks (Springer and Gold, 1989) includes a large array of sizes, life styles, and habitats that presents a great challenge to the design of MPAs for their protection. This diversity is illustrated in table III, which lists sharks of commercial importance occurring in the southern Gulf of Mexico. Table III shows that sharks can range in length from only 100 cm in the case of the sharpnose shark (*Rhizoprionodon terraenovae*), to as large as 12 m in the case of the largest living fish, the whale shark. Population dynamics also varies greatly among sharks. Growth rates can be relatively fast or slow depending on the species: the dusky smoothhound (*Mustelus canis*) reaches sexual maturity at age two, while dusky sharks take 20 years to mature. Fecundities range between about seven pups per litter in the sharpnose shark, 40-80 in the tiger shark (*Galeocerdo cuvier*), and about 300 in the whale shark. Birth size, an important parameter for juvenile survival (Branstetter, 1990), can be as small as 28 cm in the nurse shark or as large as 1 m in the dusky shark. Clearly, it is not possible to tackle the design and monitoring of MPAs for sharks without specifying which particular species of shark we are referring to.

Table III. - Life history characteristics of commercially important sharks of the southern Gulf of Mexico. (Whale sharks are not commercially important but occur in the area).

Sharks of the Southern Gulf of Mexico	Maximum total length (cm)	Female age at 1st maturity (years)	Litter size	Birth size TL (cm)	Main references	
<i>Rhizoprionodon terraenovae</i>	110	4	7	32	Parsons (1983, 1985), Branstetter (1987a).	
<i>Sphyrna tiburo</i>	117	2-3	9-12	25-40	Parsons (1987, 1993a, b), Clark & von Schmidt (1965), Alvarez (1988).	
<i>Mustelus canis</i>	152	2	20	39	Francis (1981), Compagno (1984), Pratt & Casey (1990).	
<i>Carcharhinus acronotus</i>	164	8	3-6	45-50	Clark & von Schmidt (1965), Garrick (1982), Schwartz (1984).	
<i>C. limbatus</i>	212	7	10	60	Clark & von Schmidt (1965), Killam & Parsons (1989), Branstetter (1987b).	
<i>C. brevipinna</i>	225	7	6-8	60-70	Branstetter (1981, 1987b).	
<i>C. plumbeus</i>	245	15	8-12	56-75	Casey <i>et al.</i> (1985), Springer (1960), Casey & Natanson (1992).	
<i>Ginglymostoma cirratum</i>	304		21-28	28	Compagno (1984), Pratt & Casey (1990).	
<i>Sphyrna lewini</i>	309	15	30	49	Branstetter (1987c), Klimley (1987), Pratt & Casey (1990), Chen <i>et al.</i> (1990).	
<i>Carcharhinus leucas</i>	324	18	12	60-75	Branstetter & Stiles (1987), Thorson & Lacey (1982), Garrick (1982), Pratt & Casey (1990).	
<i>C. falciformis</i>	330	12+	12	76	Garrick (1982), Branstetter (1987c), Bonfil <i>et al.</i> (1993).	
<i>C. obscurus</i>	365	20	14	100	Springer (1960), Clark & von Schmidt (1965), Natanson <i>et al.</i> (1995).	
<i>Galeocerdo cuvier</i>	550	10	43-82	51-85	Kauffman (1950), Compagno (1984), Branstetter <i>et al.</i> (1987).	
<i>S. mokarran</i>	560	?	40	50-70	Castro (1983), Compagno (1984), Pratt & Casey (1990).	
<i>Rhincodon typus</i>	1200	?	300	65	Compagno (1984), Joung <i>et al.</i> (1996).	






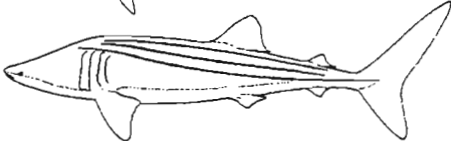
The life styles of sharks can be very specialised and diverse and will play an important role in the applicability of MPAs for management. Thus, the design of MPAs may have to be purpose-tailored depending on the life style of sharks. Considering this, it might be appropriate to classify sharks into functional groups. Here, I propose six major functional groups of sharks based on life style and the relative size of the ranges of movement of the species (Table IV). This classification does not pretend to be exhaustive and rigid. Indeed very different functional groups can perhaps be devised based on other criteria (e.g., ecological and morphological characteristics (see Compagno, 1990), or types of nursery areas). However, keeping in mind the management-oriented role of the proposed grouping, it seems to be a suitable preliminary system for aiding in MPA implementation. The six groups are: 1) bottom dwelling species with some site specificity or short range of movements such as nurse, angel (*Squatina* spp.), and wobbegong (*Orectolobus* spp.) sharks; 2) non-migratory, neritic sharks with short to medium ranges of movement such as grey reef sharks, whitetip reef sharks (*Triaenodon obesus*), blacktip reef sharks (*C. melanopterus*) and perhaps scalloped hammerhead sharks (*Sphyrna lewini*); 3) large, migratory neritic predatory sharks capable of making long-range movements such as the dusky and sandbar sharks, and perhaps the shortfin mako (*Isurus oxyrinchus*) whose habitat is both neritic and oceanic (Compagno, 1984); 4) deep-water sharks such as sixgill sharks (*Hexanchus* spp.), gulper sharks (*Centrophorus* spp.), and sleeper sharks (*Somniosus* spp.); 5) oceanic and highly migratory sharks like the blue shark (*Prionace glauca*) and cookie-cutter shark (*Isistius brasiliensis*); and 6) gigantic, planktivorous and long-ranging sharks like the whale, basking, and megamouth (*Megachasma pelagios*) sharks. Clearly, groups 1-2 are better candidates for successful MPA implementation than groups 3, 5 and 6. It is difficult to speculate about group 4 given the poor state of our knowledge of deep water sharks in general.

### Can we design effective no-take MPAs for sharks?

In this section I briefly illustrate the problems encountered in the implementation of MPAs for sharks using three hypothetical examples. The first example is the blue shark, which is a highly mobile and wide-ranging oceanic species. Figure 2, taken from Nakano (1994), shows the generalised habitat utilisation of the North Pacific Ocean by blue sharks. To protect this blue shark population through an MPA, we would have to set aside a large proportion of the North Pacific (perhaps 1/4 or 1/5 of it) to be able to include all the types of habitat needed for the blue shark throughout its life cycle. This is clearly an impossible task from the practical point of view.

The blue shark is perhaps the most extreme case of space utilisation that can be found among sharks. The second example, the spiny dogfish (*Squalus acanthias*) is a fairly small demersal shark with shorter migrations; this is the most commercially harvested elasmobranch species. This shark is caught commercially in many parts of the world but in this paper I will concentrate on the fishery off the east coast of North America. Figure 3 illustrates the migratory movements of the NW Atlantic spiny dogfish (Rago *et al.*, 1994). The stock moves from southern and deep waters in winter, towards shallower and more northerly waters in the spring. By summer, most of the stock is quite far to the north and during the autumn the cycle starts to reverse with the dogfish travelling back south and into deeper waters. In this case, it is also difficult to think of closing the entire area to fishing because other important fisheries occur there and use the same gear that catches dogfish. What is the solution? If we only close one section of the distribution range, which one should we choose? Inevitably, because of its mobility, most of the stock would

Table IV. - Major functional groups of sharks for management purposes, according to their life style and range of movement. Other criteria may be used for defining functional groups, i.e., habitat used as nursery area, or ecological and morphological characteristics (see Compagno, 1990).

Functional groups	Space utilization / Life style	
Bottom-dwelling	With some site specificity and short range of movement (e.g., nurse, angel, and wobbegong sharks)	
Neritic, non-migratory	With short to medium ranges of movement (e.g., coral reef sharks, scalloped hammerhead?)	
Neritic, migratory	With long ranges of movement (e.g., dusky, sandbar, mako? sharks)	
Deep-water	Medium ranges of movement? (e.g., sixgill, gulper, sleeper sharks)	
Oceanic	Highly migratory with long ranges of movement (e.g., blue and cookie-cutter sharks)	
Gigantic/planktivorous	With long ranges of movement (e.g., whale, basking, and megamouth sharks)	

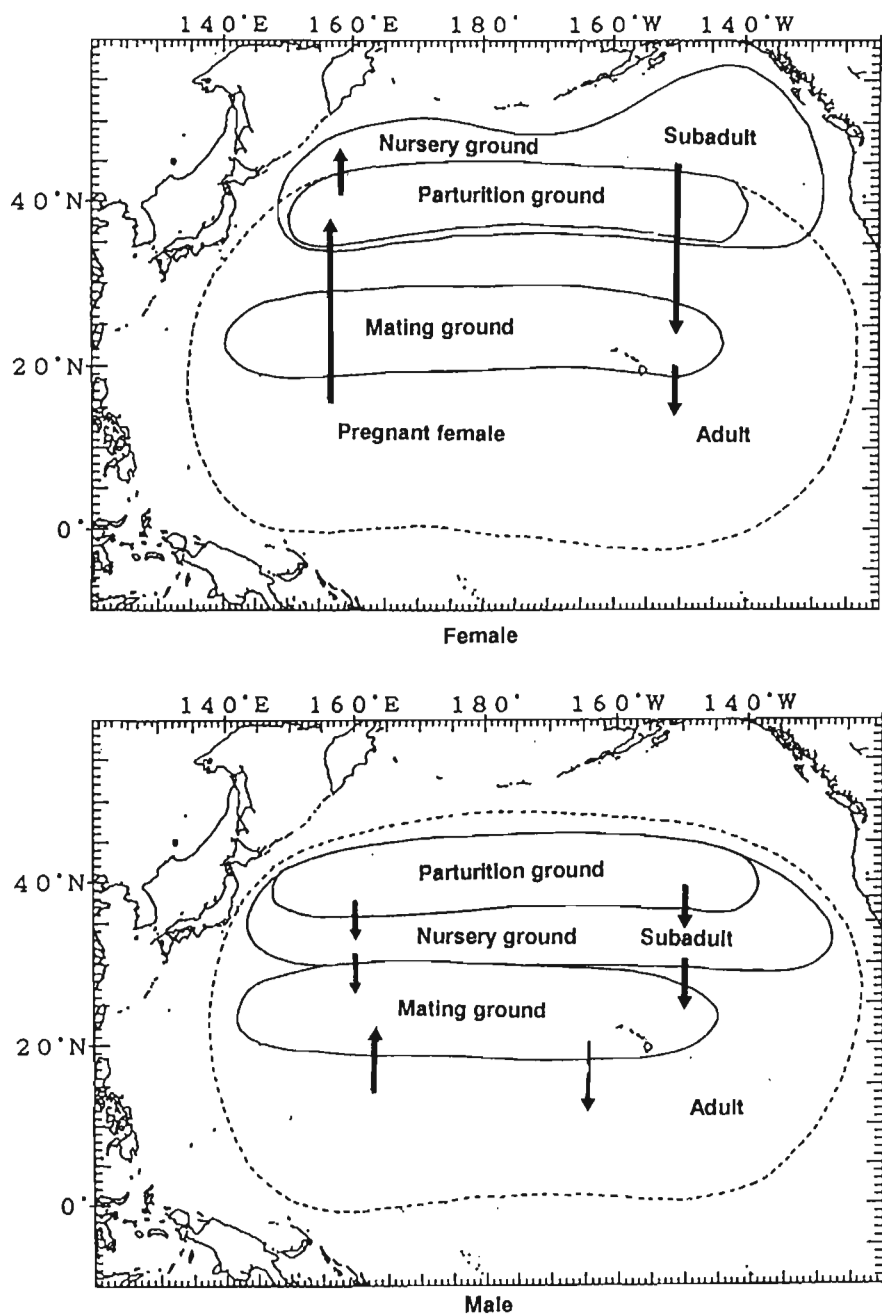


Fig. 2. - Pattern of utilisation of the North Pacific Ocean by blue sharks (taken from Nakano, 1994). Implementing an effective no-take MPA for this population would mean setting apart an unthinkable large portion of the North Pacific.

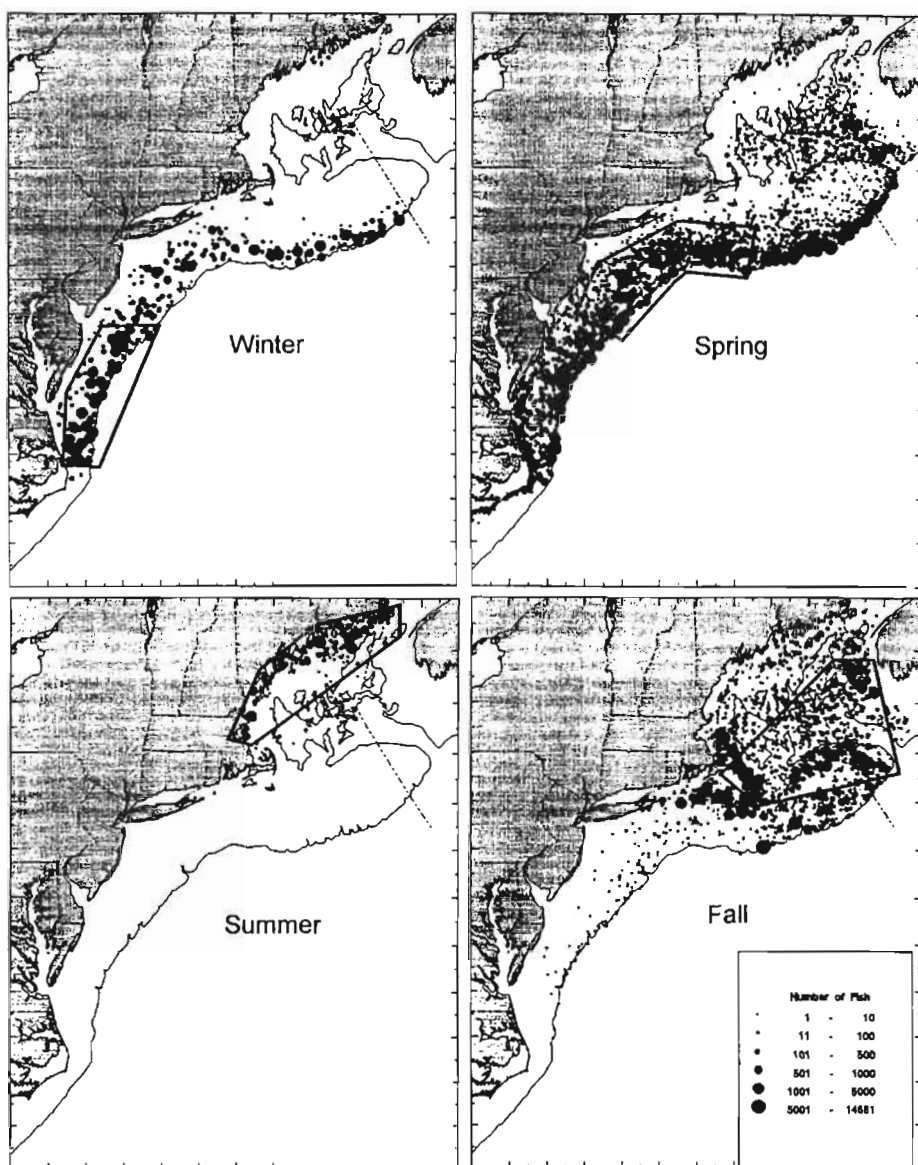


Fig. 3. - Seasonal distribution of spiny dogfish in the east coast of the USA and Canada (modified from Rago *et al.*, 1994). The black polygons show suggested rolling MPAs that could be used to protect part of the stock from fishing year-round.

be vulnerable to fishing for a large portion of the year. Perhaps, the best solution would be to place a dynamic or 'rolling' MPA that moves along with the dogfish stock during its migration. In all likelihood, this is a very difficult if not impossible scenario to implement, not only because of the logistic challenges it presents for fishing and surveillance



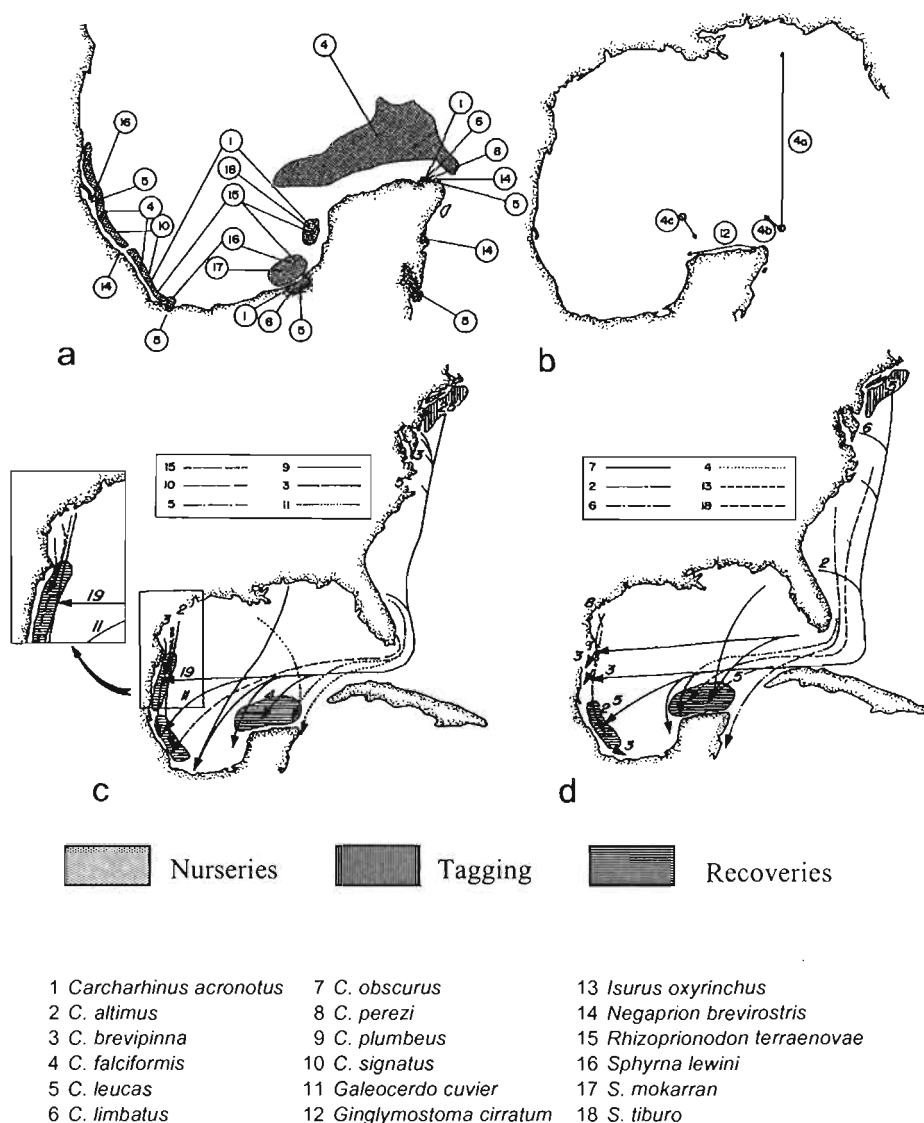


Fig. 4. - These four maps illustrate the complexity of MPA implementation for sharks in the southern Gulf of Mexico. **a**: Nursery areas. Possible migration patterns can be inferred from recapture of tagged sharks: **b**: Sharks tagged by the Instituto Nacional de la Pesca, Yucalpeten Lab Shark Tagging Program; **c**, **d**: Sharks tagged by NMFS Cooperative Shark Tagging Program. Numbers encircled in **a**, **b** and those in the key to lines in **c** and **d** correspond to the species listed on the bottom part of the figure. The numbers associated to lines in **c** and **d** are number of sharks that were tagged (origin of arrows) or recovered (near head of arrows); arrows without numbers indicate a single shark. (From Bonfil, 1997).

operations but also because of the market and other social and economic factors this measure would upset. This type of setup was unsuccessfully proposed in the southern shark

fishery of Australia (Terry Walker, Marine and Freshwater Resources Institute, Victoria, Australia, pers. comm. 1997). For a rolling MPA to be successful, managers and fishermen will have to work very tightly together in an unusually co-operative atmosphere.

The last example deals with the multispecies shark fisheries in the southern Gulf of Mexico. Here, the complications of using MPAs for sharks are tremendous. There are up to 25 different species taken in the fishery (Bonfil, 1997) although only about 15 are illustrated in figure 4. Even trying to close identified nursery areas to protect the juveniles implies a major task given that many coastal low-income communities depend on fishing in those areas for subsistence. Also, the migratory routes of some of these species are quite long as shown by the recaptures from the NMFS cooperative shark tagging programme. It is almost impossible to design effective MPAs for fisheries management under these circumstances.

## DISCUSSION

The general principle of protecting **all life-stage** for an MPA to be effective presents a problem for the successful implementation of MPAs for sharks. From this point of view, the potential usefulness of MPAs as sole measure for most shark management and protection issues is limited. This is specially true for species such as the basking, blue and whale sharks, which have extensive migrations. However, currently it also seems difficult to implement successful MPAs as the main solution for the management of most other shark species. Our knowledge of key aspects of shark biology and ecology such as migratory routes, rates of movement, nursery areas, and space utilisation, are not nearly enough to guarantee proper design of MPAs that would support viable populations in most cases. A possible important exception to this rule of thumb is the implementation of MPAs for freshwater elasmobranchs. Most of these species are facing increasingly threatening pressure from human development in their narrow and localised distribution ranges and could benefit greatly from total protection in the mostly tropical river systems where they occur.

While it is true that the benefits of no-take MPAs are not clearly evident for organisms that have limited site specificity, such as the long-range migratory sharks, it is possible that no-take MPAs can be an effective way to support and complement the conservation of some shark and ray species by providing a haven during key parts of their life-cycles. Coastal nursery areas, mating grounds, and some specific feeding grounds where species might congregate are excellent candidates for MPAs. Obviously, the effectiveness of these sites will always depend on suitable complementary management and protection measures such as controlled harvest regimes throughout the entire range of the stock outside the MPA. If MPAs cannot alone be the solution for shark management, they can surely be a very effective aid for management.

If we are to advance in the design of MPAs for sharks, research efforts should be directed chiefly (but not exclusively) towards defining the size of home ranges, migratory routes and timings, and the rates of movement inside and outside potential marine reserves. Several types of tagging studies will have to be implemented to achieve this.

The charismatic image that sharks are gaining in recent times can be very favourable for the political process involved in the instigation of MPAs. In this sense, species such as the great white shark or the whale shark can become flag species that could positively impact a wide range of species from the extended benefits of the protection of entire ecosystems.

**Acknowledgements.** - Merry Camhi, Colin Simpendorfer and Terry Walker provided valuable information on the status of protection of sharks in each of their geographic regions. The suggestions of two anonymous reviewers for improving the manuscript are greatly appreciated.

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**A SYNOPSIS OF THE DEEP-SEA GENUS *BENTHOBATIS* ALCOCK,  
WITH A REDESCRIPTION OF THE TYPE SPECIES  
*BENTHOBATIS MORESBYI* ALCOCK, 1898  
(CHONDRICHTHYES, TORPEDINIFORMES, NARCINIDAE)**

by

Marcelo R. de CARVALHO (1)

**ABSTRACT.** - The electric ray genus *Benthobatis* Alcock, 1898 is reviewed and found to contain four continental slope species, two of which were previously described. *Benthobatis moresbyi* Alcock, 1898, the type-species, is known from five specimens from both sides of the Arabian Sea (off southwestern India and southern Yemen) and western Indian Ocean (continental slope of Somalia). *Benthobatis marcida* Bean & Weed, 1909, of which *B. cervina* Bean & Weed, 1909 is a junior synonym, is known from abundant material from the southeastern continental slope of the United States, northern Cuba and the Bahamas. Two undescribed species of *Benthobatis* exist, one from the southwestern Atlantic ocean (continental slope of southern Brazil) and one from the South China Sea (off southwestern Taiwan). *B. moresbyi* is redescribed based on material from all currently known localities, and a lectotype is designated. *B. moresbyi* is diagnosed by a combination of characters, including its uniform dark brown dorsal and ventral coloration, elongated snout area, highly oval disc, fleshy dorsal fins with long bases, very small interdorsal distance, and low and elongated caudal fin positioned very close to the second dorsal fin. All four species of *Benthobatis* are diagnosed and illustrated, and a key for their identification is provided. Prior to this account, no new information on *B. moresbyi* has appeared in almost 90 years.

**RÉSUMÉ.** - Révision du genre *Benthobatis* Alcock, et nouvelle description de son espèce-type *Benthobatis moresbyi* Alcock, 1898 (Chondrichthyes, Torpediniformes, Narcinidae).

La révision des raies électriques du genre *Benthobatis* a permis la reconnaissance de quatre espèces de la pente continentale, dont deux étaient déjà décrites. L'espèce-type *Benthobatis moresbyi* Alcock, 1898 est connue par cinq spécimens qui proviennent du large de l'Inde du sud-ouest et du Yémen du sud ainsi que de l'Océan Indien Ouest (pente continentale de la Somalie). De nombreux spécimens de *Benthobatis marcida* Bean & Weed, 1909, dont *B. cervina* est synonyme proviennent de la pente continentale du sud-est des États-Unis, du nord de Cuba et des Bahamas. Il existe deux espèces non décrites de *Benthobatis*, l'une provenant du sud-ouest de l'Océan Atlantique (pente continentale du Brésil) et l'autre du sud de la mer de Chine (sud-ouest de Taiwan). *B. moresbyi* est redécrite sur la base de matériel provenant de toutes les localités connues et un lectotype est désigné. La diagnose de *Benthobatis moresbyi* se fonde sur la combinaison de plusieurs caractères dont la coloration (dorsale et ventrale) brun foncé, le museau allongé, un disque de forme nettement ovale, les nageoires dorsales à base allongée, une distance interdorsale très petite, la nageoire caudale basse et allongée très proche de la deuxième nageoire dorsale. La diagnose et l'illustration des quatre espèces de *Benthobatis* sont présentées et une clé d'identification est proposée. Aucune information nouvelle concernant *B. moresbyi* n'avait été publiée depuis près de 90 ans.

**Key-words.** - Torpediniformes, Narcinidae, *Benthobatis*, *B. moresbyi*, *B. marcida*, Taxonomy, Lectotype, Electric rays, Continental slope.

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The electric ray genus *Benthobatis* was described by Alcock (1898) for the new species *B. moresbyi*, based on three specimens trawled by the R.I.M.S. "Investigator" in 1897 from deep waters off the southwestern coast of India. Following the first illustration of this species (Alcock, 1899a; reproduced here as Fig. 1), both Lloyd (1907) and Brauer (1908) extended its range to include the western Arabian Sea (continental slope of Yemen) and western Indian ocean (continental slope of Somalia), respectively. Specimens have not been collected since 1906. Annandale (1909) briefly mentions *Benthobatis* and provides an illustration of its mouth and teeth based on material previously collected. No new information has been published on *B. moresbyi* since.

Bean and Weed (1909) described the new species *Benthobatis marcida* and *B. cervina* on the basis of two specimens from deep waters of the western north Atlantic ocean (continental slope of the eastern coast of Florida). *B. cervina* was subsequently synonymized with *B. marcida* (Bigelow & Schroeder, 1953), which is now known to occur in various localities in the western north Atlantic, Straits of Florida, off Cuba (Nicholas and Old Bahama Channels) and the Bahamas, in depths of 274 to 923 m.

*Benthobatis* is a rare deep-sea genus that remains relatively poorly known, especially in the Indo-Pacific region. However, recent collecting efforts have increased its range to include the southwestern Atlantic ocean<sup>(2)</sup> and southwestern coast of Taiwan (Chen and Chung, 1971; Shen, 1984a; Chen and Joung, 1993; Compagno *et al.*, unpubl. data). Both new occurrences constitute new species of *Benthobatis*, to be named elsewhere. Given the paucity of information available for the type-species, *B. moresbyi* Alcock, a revision of the genus was initiated in conjunction with revisionary studies currently being undertaken on all torpediniform genera. As both new species are in the process of being described, it is pertinent to have more information available for the type-species, which is redescribed below. The following account includes all species of *Benthobatis*, but focuses primarily on *B. moresbyi*. All available specimens of *B. moresbyi* were examined (including one type-specimen, designated as lectotype below), with the exception of two of Alcock's original specimens that remain in Calcutta.

## MATERIAL AND METHODS

Abbreviations for the 43 measurements taken on specimens of *B. moresbyi* are as follows (a more complete description of measurements can be found in an upcoming revision of *Narcine*): Total length (TL); disc width (DW); disc length (DL); preocular snout length (PCSL); preoral snout length (POSL); prenasal snout length (PNSL); snout to maximum disc width (SDW); interorbital distance (IOD); eye length (EL); interspiracular distance (ISD); spiracle length (SL); spiracle width (SPW); mouth width (MW); upper exposed tooth band width (UTBW); lower exposed tooth band width (LTBW); nasal curtain width (NCW); nasal curtain length (NCL); distance between nostrils (DBN); distance between first gill openings (DFGO); distance between last gill openings (DLGO); branchial basket length (BBL); pelvic fin length (PFL); pelvic fin width (PFW); length of anterior lobe of pelvic fin (ALPF); length of posterior lobe of pelvic fin (PLPF); tail width (TW); height of first dorsal fin (HFD); length of first dorsal fin (LFD); height of second dorsal fin (HSD); length of second dorsal fin (LSD); length of dorsal lobe of caudal fin (LDLC); length of ventral lobe of caudal fin (LVLC); height of dorsal lobe of caudal fin (HDLC);

(2) This occurrence was originally reported by C. Vooren and G. Rincón in an abstract of the VI Reunião do Grupo de Trabalho sobre Pesca e Pesquisa de Tubarões e Raias no Brasil (1993, p. 230).

height of ventral lobe of caudal fin (HVLC); height of caudal fin (HC); distance between first and second dorsal fins (DBD); distance between second dorsal and caudal fins (DSDC); snout to cloaca length (SCL); cloaca to caudal fin tip (CLCF); snout to first dorsal length (SFD); electric organ length (EOL); electric organ width (EOW); clasper length (CL). It was not possible to take all measurements on all specimens of *B. moresbyi*, as specimens were partially dissected previously, or distorted beyond accuracy for a particular measurement<sup>(3)</sup>. Measurements are only provided for specimens of *B. moresbyi*, as these are the primary concern of this paper (Table I). Institutional abbreviations follow Leviton *et al.* (1985). Material examined is listed separately for each species.

Meristic data for the three examined specimens of *B. moresbyi* are as follows (summarized in Table II): number of propterygial radials (PROP); mesopterygial radials (MESO); metapterygial radials (META); total pectoral radials (TPR = PROP + MESO + META); pelvic radials (PR; first enlarged radial counted as one); first dorsal fin radials (FDR); second dorsal fin radials (SDR); dorsal caudal fin radials (DCR); ventral caudal fin radials (VCR); total caudal fin radials (TCR = DCR + VCR); upper exposed vertical tooth rows (UTR); lower exposed vertical tooth rows (LTR); trunk vertebral centra (TC = first distinguishable vertebral centrum in synarcual cartilage to anterior border of pelvic girdle); precaudal centra (PC = centrum from anterior border of pelvic girdle to caudal fin origin); caudal centra (CC = centrum from origin of caudal fin to last distinguishable centrum in caudal fin tip); total vertebral centra (TV = TC + PC + CC); ribs (R).

The description of *B. moresbyi* is based on all examined specimens, not just the lectotype herein designated, but special reference is made to a particular specimen when appropriate. The brief account on *B. marcida* is based more on the type specimen. The sections "Referred specimens" list only non-type material examined. Terminology for the anatomical description of *B. moresbyi* follows Miyake (1988), Nishida (1990) and Carvalho (unpubl. data).

### BENTHOBATIS ALCOCK

*Benthobatis* Alcock, 1898: 144 (original description, not figured); Garman, 1913: 294 (recognized as valid, description compiled); Fowler, 1941: 339 (description compiled); Bigelow and Schroeder, 1953: 126 (recognized as valid, compilation, partial revision); Misra, 1969: 216-218 (recognized as valid, description compiled); Compagno, 1973: 27 (recognized as valid, listed in a classification); Nelson, 1976: 43 (listed only); Fechhelm and McEachran, 1982: 173, 200, 202-203 (recognized as valid, anatomical comparison and illustration); Nelson, 1984: 61 (listed only); Miyake, 1988 (accepted as valid); Eschmeyer and Bailey, 1990: 57 (recognized as valid); Eschmeyer, 1990: 437 (listed in a classification); Nelson, 1994: 59 (listed only); McEachran *et al.*, 1996: 80 (recognized as valid, listed in a classification); Eschmeyer and Bailey, 1998: 1862 (recognized as valid); Eschmeyer, 1998: 2451 (listed in a classification).

*Synonymy.* - None.

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(3) Caution is necessary when using morphometric characters to identify species of *Benthobatis*, as preserved specimens can easily be deformed from their original state. This is primarily because electric rays lack dermal hard structures, such as denticles and spines, which may help maintain body shape and rigidity when preserved (in preservative, electric rays may have flabby skin which is easily detached from underlying tissues, especially in *Benthobatis*). Also, *Benthobatis* occurs in deep waters, so bloating of the skin may happen as a result of dredging specimens from the ocean floor. Even with this constraint, positions of fins and relative proportions proved very useful in diagnosing species because there are obvious differences among them.

*Type-species.* - *Benthobatis moresbyi* Alcock, 1898, by original designation and monotypy.

### Diagnosis

Narcinid electric rays with elongated oval to rounded discs; snout greatly to moderately elongated, supported by rostrum with great antero-lateral expansion; extremely reduced and presumably non-functional eyes, not readily visible dorsally anterior to spiracles; a small pore dorsal to embedded eyes sometimes present; nostrils not subdivided in two distinct apertures by stiff tissue; spiracles small, generally wider than long, with smooth borders and without elevated rims; pelvic fins adjoining lateral aspect of tail throughout entire length, not leaving free lobe posteriorly; seemingly encasing claspers in immature specimens; pelvic fins contacting tail posterior to clasper extremities; pelvics not united to each other posteriorly to form "apron"; anterior portion of pelvic fins greatly to moderately expanded laterally; tail abruptly narrower than trunk; tail thin and relatively long, longer than disc (tail measured from cloaca); lateral tail folds inconspicuous, in the form of barely noticeable small ridges; highly oval and elongated caudal fin, lower margin of which is continuously curved, with very low inferior lobe; dorsal fins moderately tall, with very long to moderate bases, and with posterior margins contacting tail without leaving great prominent free lobe posteriorly; teeth in very few exposed rows, rows faintly noticeable when mouth is closed, portion of exposed tooth band devoid of teeth; electric organs only barely noticeable externally in dorsal or ventral views; skin seemingly a bit loose and reduced superficial calcification of skeleton, accounting in part for general flabbiness of body.

### Included species

*Benthobatis moresbyi* Alcock, 1898 (Indo-Pacific), *B. marcida* Bean & Weed, 1909 (northwestern Atlantic), *Benthobatis* sp.1 (southwestern Atlantic), *Benthobatis* sp.2 (South China Sea).

### Remarks

*Benthobatis* is easily distinguished from genera of torpedinid (*Torpedo* Houttuyn, 1774), hypnid (*Hypnos* Duméril, 1865) and narkid (*Narke* Kaup, 1826; *Heteronarke* Regan, 1921; *Temera* Gray, 1831; *Typhlonarke* Waite, 1909) electric rays by the combination of: a protractile but not widely distensible mouth; nasal curtain wider than long, not intensely studded with sensory pores along most of its length; nostrils circular, not slit-like; teeth with a single cusp and ovoid bases; tail relatively long, longer than disc or more or less same length; presence of two dorsal fins of about same size. In addition, *Benthobatis* presents a series of anatomical characters shared with other narcinids (*Narcine* Henle, 1834; *Discopyge* Heckel, 1846; *Diplobatis* Bigelow & Schroeder, 1948), that further separates it from non-narcinid torpediniform genera: broad, flattened trough-shaped rostrum (much more antero-laterally expanded in all species of *Benthobatis* than in any other narcinid thus far examined); elongated and relatively broad precerebral fontanelle, associated with the particular rostral morphology; laterally branching antorbital cartilages (degree of branching variable among narcinid genera, and even within species of *Narcine*); relatively straight orbital area (curving mesially in other torpediniform families); at least two pairs of relatively strong labial cartilages; presence of triangular protuberances (nasal capsule "horns") on dorsal aspect of nasal capsules.

The majority literature of records of *Benthobatis* are compilations from a few primary sources, such as the original species descriptions of Alcock (1898, and illustration



of 1899a) and Bean and Weed (1909). The only additional information given for the genus after these authors, and before the account of Bigelow and Schroeder (1953), are the range extensions for *B. moresbyi* by Lloyd (1907) and Brauer (1908), for *B. marcida* by Chace (1940), and an illustration of the mouth and teeth of *B. moresbyi* by Annandale (1909). Bigelow and Schroeder (1953) provided much new information for *B. marcida*, but no new information for *B. moresbyi* has appeared after Annandale (1909).

*Benthobatis* is a poorly known, deep-sea genus of electric ray, occurring in cold waters of tropical and sub-temperate areas, in depths of 274 to 1071 m. No other genus of Torpediniformes has been captured at depths of 1000 meters or more (however, not all species of *Benthobatis* occur at this depth either). *Heteronarce* Regan, 1921, a narkid, has been collected in areas close to where *Benthobatis* is known to occur (southwestern coast of India, western Arabian Sea), but from waters not as deep (ranging from 80 to 305 m only). The bathymetric distribution of *Benthobatis*, requiring special collecting efforts, may account for the paucity of specimens of at least the type-species, *Benthobatis moresbyi*. *B. marcida*, from the western North Atlantic, is more abundant in collections. *B. marcida* has been collected from shallower waters (from 274 to 910 m) than *B. moresbyi*, and many specimens are usually sampled together. This is also true for the undescribed Brazilian species of *Benthobatis* (referred to here as *Benthobatis* sp.1).

Given that the species of *Benthobatis* are somewhat geographically restricted, that they occur in deep waters and that specimens have been collected from distant localities, the number of species may be higher than the four currently recognized.

### Etymology

*Bentho*, from the Greek "benthos", meaning depth of the sea or sea-bottom; *batis*, a Greek word for skate or ray. Gender feminine.

### Vernacular names

Blind numbfishes, deep-sea numbfishes, blind electric rays.

### Key to species of *Benthobatis*

- 1a. Uniform light brown dorsally; white to yellowish-white ventrally; western North Atlantic ..... *Benthobatis marcida*
- 1b. Dark brown to blackish-brown dorsally; dark brown or white ventrally ..... 2
- 2a. Caudal fin less than half length of tail as measured from posterior tips of pelvic fins; dorsal fin length equal or subequal to dorsal fin height; ventrally white with dark posterior outline to ventral margin of pelvic fins; western South Atlantic ..... *Benthobatis* sp.1
- 2b. Caudal fin about half length of tail as measured from posterior tips of pelvic fins; dorsal fins longer than tall; dorsal and ventral coloration very dark brown or blackish-brown ..... 3
- 3a. Distance between dorsal fins less than length of their individual bases; dorsal fins about twice as long as tall; Arabian Sea, western Indian ocean ..... *Benthobatis moresbyi*
- 3b. Distance between dorsal fins greater than length of their individual bases; dorsal fin height about three-fourths in dorsal fin length; South China Sea ..... *Benthobatis* sp.2

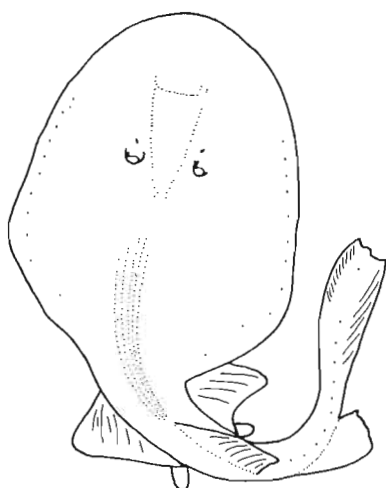


Fig. 1. - Alcock's (1899a) original outline rendering of *B. moresbyi*, clearly depicting generic and specific diagnostic characters such as lack of eyes, elongated snout, shape and position of dorsal fins, and elongated caudal fin.

Table I. - Measurements taken on specimens of *Benthobatis moresbyi*. Total length given in mm, all other measurements expressed as percentages of total length. Abbreviations are explained in Material and Methods.

	BMNH 1898.7. 13.22 (lectotype)	BMNH 1909.7. 12.7	ZMB 17413		BMNH 1898.7. 13.22 (lectotype)	BMNH 1909.7. 12.7	ZMB 17413
1. TL (mm)	351.2	120.0	392.4	23. PFW	20.7	21.5	37.1
2. DW	38.4	36.1	37.9	24. ALPF	10.7	11.6	11.0
3. DL	43.6	42.5	43.5	25. PLPF	13.5	8.5	24.3
4. PCSL	17.4	17.4	13.6	26. TW	13.9	15.2	21.7
5. POSL	15.6	15.6	12.7	27. HFD	5.5	2.3	6.6
6. PNSL	15.8	13.4	9.7	28. LFD	9.8	7.5	8.9
7. SDW	32.0	27.4	29.6	29. HSD	5.6	3.1	6.0
8. IOD	7.2	6.3	7.9	30. LSD	11.1	7.2	9.4
9. EL	0.5	-	0.4	31. LDLC	19.4	-	18.4
10. ISD	5.4	6.5	6.4	32. LVLC	20.1	-	18.5
11. SPL	2.1	1.8	2.3	33. HDLC	1.3	-	1.5
12. SPW	2.1	1.5	2.3	34. HVLC	2.3	-	1.1
13. MW	5.4	5.2	7.0	35. HC	5.6	-	5.4
14. UTBW	1.6	-	2.2	36. DBD	2.2	5.3	2.9
15. LTBW	1.2	-	1.7	37. DSDC	1.7	-	2.9
16. NCW	-	-	5.1	38. SCL	48.6	47.3	47.1
17. NCL	1.6	-	1.7	39. CLCF	49.8	50.8	49.7
18. DBN	5.3	4.6	4.6	40. SFD	54.4	57.2	50.0
19. DBFGO	11.0	10.8	14.0	41. EOL	17.2	24.3	22.5
20. DBLGO	8.6	7.8	12.9	42. EOW	8.9	7.4	7.0
21. BBL	8.6	9.8	10.8	43. CL	11.2	7.0	-
22. PFL	15.6	12.0	19.9				

**BENTHOBATIS MORESBYI ALCOCK**

Figs 1-4A, 5; Tables I-II

*Benthobatis moresbyi* Alcock, 1898: 143 (name only), 145 (original description, not figured; southwestern India); Alcock, 1899a: pl. 26, fig. 1 (outline illustration); Alcock, 1899b: 18 (repeat of original description, almost verbatim; registration numbers given); Alcock, 1899c: 83 (name only); Lloyd, 1907: 4 (continental slope of Yemen); Brauer, 1908: 9-10 (continental slope of Somalia); Annandale, 1909: 4, 46, pl. IIIA, figs 5, 5a (brief account; illustration of mouth and teeth); Lloyd, 1909: 145 (compilation); Bean and Weed, 1909: 677, 679, 680 (compilation); Garman, 1913: 294 (compilation); Fowler, 1941: 339 (compilation); Misra, 1949: 44 (compilation, synonymy); Misra, 1952: 133 (compilation); Bigelow and Schroeder, 1953: 126, 127 (compilation); Misra and Menon, 1958: 78 (compilation); Menon and Yazdani, 1968: 98, 168, 180 (name only); Misra, 1969: 216-218 (description; illustration compiled); Menon and Rama Rao, 1970: 377 (name only); Eschmeyer *et al.*, 1998: 1121 (recognized as valid, type-specimens indicated); Eschmeyer, 1998: 2182 (listed in a classification).

*Type-specimens.* - BMNH 1898.7.13.22 (lectotype, herein designated), off Travancore coast, southwestern India (7°17'30"N, 76°54'30"E; coordinates from Eschmeyer *et al.*, 1998: 1121), 786.9 m, 351 mm TL adult male, collected 19 Oct. 1897 by the RIMS "Investigator" (ex-Indian Museum specimen, Calcutta, formerly registered as ZSI 233/1); ZSI 232/1 and ZSI 234/1 (paralectotypes, not examined), collected with lectotype, adult male of approximately 350 mm TL and "young" specimen (sex and TL unknown).

Table II. - Counts of meristic features taken on specimens of *Benthobatis moresbyi*. Certain counts could not be taken on available radiographs. Abbreviations are explained in the Material and Methods section. For specimen ZMB 17413, no distinct mesopterygium and metapterygium were observed on radiographs. However, nine radials were counted posterior to propterygium.

	BMNH 1898.7.13.22 (lectotype)	BMNH 1909.7.12.7	ZMB 17413
1. PROP	—	—	10
2. MESO	—	—	— <sup>1</sup>
3. META	—	—	— <sup>1</sup>
4. TPR	—	—	19
5. PVR	14	14	16
6. FDR	—	5	6
7. SDR	8	5	—
8. DCR	—	—	30
9. VCR	26	—	30
10. TCR	—	—	60
11. UTR	18	9	18
12. LTR	19	—	14
13. TC	19	19	19
14. PC	58	60	56
15. CC	38	36	38
16. TV	115	115	113
17. R	—	—	6

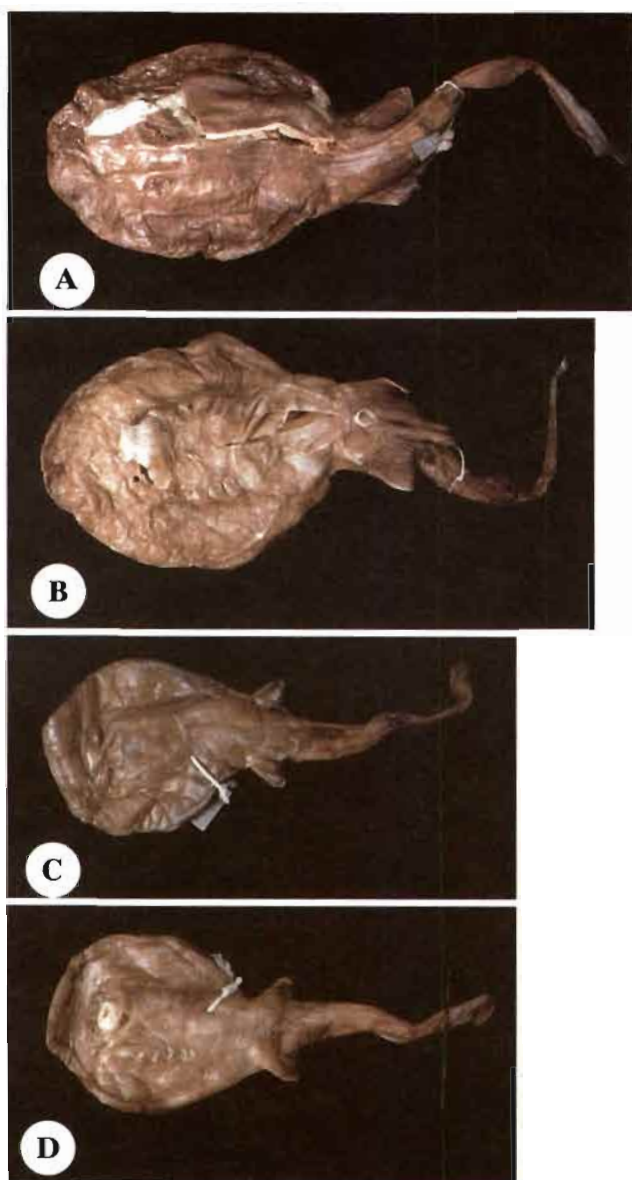


Fig. 2. - *Benthobatis moresbyi* Alcock, 1898. A and B: dorsal and ventral views, respectively, of lectotype (BMNH 1898.7.13.22, male 351 mm TL). C and D: dorsal and ventral views, respectively, of BMNH 1909.7.12.7 (male 120 mm TL), from continental slope of Yemen.

### Diagnosis

A species of *Benthobatis* distinguished from congeners by the combination of dark brown dorsal and ventral surfaces; ventral surface entirely dark; elongated snout,

usually more than one-third in disc length (preocular snout region 40% in disc length in lectotype); disc oval, longer than wide; dorsal fins with long, fleshy bases, bases much longer than height of dorsal fins (dorsal fin length 50 to 55% of fin height); origin of first dorsal well anterior to posterior tip of pelvic fins, close to mid pelvic length; dorsal fins close together, interdorsal space less than length of dorsal bases; distance between second dorsal fin and caudal fin much smaller than length of base of second dorsal fin; caudal fin extremely elongated, reaching almost one-half tail length as measured from posterior tips of pelvic fins, caudal fin length 40.4% of distance between cloaca and caudal fin tip.

### Description

Measurements for examined specimens are given in table I, and meristics in table II.

*External morphology.* - Skin seemingly very loose on body, giving specimens a flabby, soft aspect. Disc length less than half in total length, disc widest at close to two-thirds of disc length; disc oval, with greatly elongated snout (more pronounced in lectotype), preocular snout region more than one-third in disc length (about 40% in lectotype); snout region rounded to angular anteriorly; disc not overlapping pelvic fins posteriorly, contacting trunk region more or less at same level of pelvics, and anterior to origin of pelvics; eyes very minute, visible dorsally as eye "spots", embedded within integument, underlying skin and probably not functional; small pore of unknown depth and function lying dorsal to eye; spiracles separated from eyes by a small distance; spiracles wider than long, with circular posterior borders, and without distinct anterior margins; small pseudobranch present within spiracles; interocular and interspiracular distance slightly less than one-half preorbital distance.

Preorbital distance greater than prenasal distance, and more or less equal to preoral distance; mouth width about one-seventh of disc length (less so in Somalian specimen, which has a slightly distorted, shrunken disc); nostrils small and circular, not slit-like, directly anterior to mouth corners; in ZMB 17413 nostrils with small projection extending posteriorly from anterior margins (Fig. 3C); internasal distance almost equal to mouth width; nasal curtain much wider than long, extending posteriorly almost to mouth, and not studded with pores posterior to nostrils; mouth greatly and artificially protruded in lectotype (less so in BMNH 1909.7.12.7). Teeth in 18/19 total rows (exposed and not exposed) in lectotype; 9 exposed upper rows in BMNH 1909.7.12.7 (lower tooth band heavily damaged, but with as many as 11 total rows counting from radiograph); 18/14 exposed rows in specimen from off Somalia; teeth with wide, ovoid bases and only moderately elongate and dull cusps; all teeth unicuspid, similar in upper and lower tooth bands; middle rows of lower tooth band with teeth missing in lectotype; teeth in central, not exposed, rows with longer cusps that still do not overlap teeth situated in next posterior row; teeth set in quincunx arrangement. Gill openings small, semicircular; first gill slits situated close to mid disc length ventrally, last gill slits at posterior one-fourth of disc, close to coracoid bar; distance between first gill slits greater than distance between last gill slits; distance between first and last gill slits about equal to one-fifth disc length.

Pelvic fins greatly expanded laterally at anterior portion, reaching up to same width as disc when distended in Somalian specimen (disc is shrunken), but close to one-half width of disc in specimen from off Yemen and lectotype; pelvic fins triangular in outline, angular laterally, with more or less straight posterior margins that connect with lateral aspect of tail throughout entire length leaving no free posterior lobe; pelvic fin

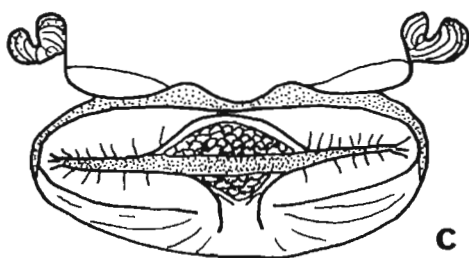
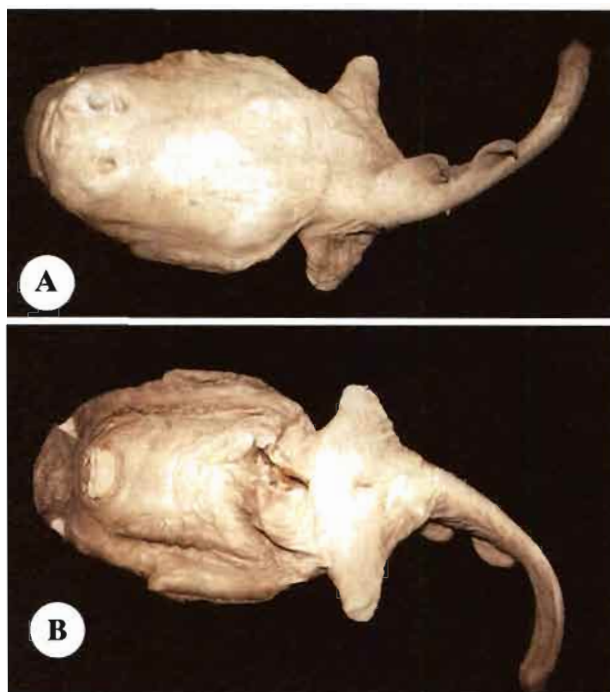


Fig. 3. - *Benthobatis moresbyi* (female 392.4 mm TL) from continental slope of Somalia (ZMB 17413). A: dorsal view. B: ventral view. C: detail of mouth and nostrils (mouth less protruded, shown in more natural position).

length more than one-third disc length; pelvic fins extend posteriorly dorsal to clasper in lectotype and in specimen from off Yemen, and terminate posterior to clasper tip; posterior margin of pelvics straight to slightly concave, continuing only as a slight ridge towards posterior tip. Cloaca in lectotype highly expanded. Distance between clasper tip and cloaca in adult male roughly one-fourth disc length; clasper groove curved towards mid-line posteriorly at area of clasper glans; both pseudosiphon and pseudopora visible in dorsal view.

Dorsal fins fleshy, more so at bases, with a loose skin covering. First dorsal fin originates at close to one-half of pelvic fin length; dorsal fins rather low, with curved, sloping anterior margins and posterior margins inflecting slightly anteriorly close to tail; length of base of dorsal fins much greater than height (first dorsal almost twice as long as high, second dorsal exactly twice as long as high); dorsal fins about equal in height, but second dorsal has longer base; distance between dorsal fins very small (almost equal to spiracle length); distance between second dorsal and caudal fin even smaller than

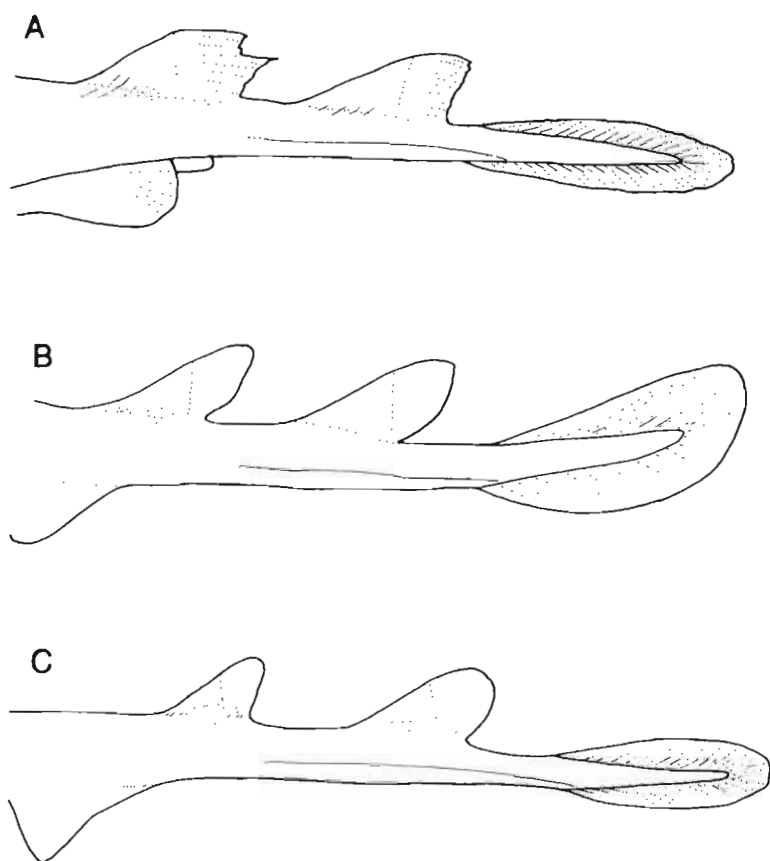


Fig. 4. - Lateral view of tail region, demonstrating differences in position and configuration of dorsal and caudal fins. A: *Benthobatis moresbyi*. B: *B. marcida*. C: *Benthobatis* sp.2.

distance between dorsals; dorsal fins with sub-triangular tips (except in lectotype, which lacks the tip of first dorsal fin). Caudal fin long, close to 2.4% length of tail as measured from cloaca to caudal fin tip; caudal fin low, its height when measured from superior to inferior margins about equal to height of dorsal fins. Lateral tail ridges barely noticeable, precise origin anteriorly difficult to discern. On lectotype, pores of lateral-line canal extending posteriorly on lateral aspect of tail just ventral to dorsal fins, deflecting downwards posterior to second dorsal fin, and continuing beyond caudal peduncle towards tip of caudal fin.

**Coloration.** - Dorsal and ventral coloration entirely dark brown, with patches of darker brown on sides of disc, posterior region of ventral pelvic fins, tail and caudal fin. Alcock (1898) describes *Benthobatis moresbyi* as being « purplish-black » and states that on one specimen « part of the tips of the second dorsal and caudal fins are white » (1898: 145). This specimen is presumably one of the paralectotypes that remain in the ZSI, Calcutta, as the lectotype does not have fins with white tips. Alcock also mentions the presence of small white pores (just smaller than eyes) scattered on the disc and disc periphery (no longer visible on lectotype). Alcock probably examined the specimens

before they were placed in preservative, as his account of their coloration seems to be based on fresh material. *B. moresbyi* was « dredged since 1896 » (Alcock, 1898: 136), therefore he had access to them shortly after collected.

*Skeletal anatomy.* - Skeletal calcification reduced, generally without clear areas of reinforcing superficial calcification, except perhaps on jaws and synarcual cartilage. Rostral portion of neurocranium wide anteriorly, tapering slightly from snout tip towards nasal capsules. Morphology of anteriormost region of rostrum not clear in radiographs, but rostral tip rounded anteriorly, without a large median notch which is present in some specimens of *B. marcida*. Precerebral fontanelle very long, with highly curved posterior border anterior to internasal space; difficult to discern if circular "rostral fontanelle" (precerebral fontanelle of some authors) is present anterior to actual precerebral fontanelle. Fronto-parietal fontanelle beginning slightly posterior to precerebral fontanelle and extending caudally to just anterior to origin of hyomandibulae. Post-rostral region of neurocranium widest at nasal capsules. Nasal capsules wide and short, articulating with antorbital cartilages laterally. Antorbital cartilages thin (stouter on Somalian specimen), branching distally into two thin segments at about one-fourth distance from nasal capsules to antero-lateral borders of disc; small, inconspicuous, posteriorly directed segment of antorbitals present close to articulation of antorbitals with nasal capsules; antorbitals apparently not contacting lateral aspects of rostrum anteriorly. Interorbital distance small, close to one-fourth length of neurocranium from occipital segment to nasal capsules.

Hyomandibulae stout at bases, originating from otic region of neurocranium, and tapering distally to meet corners of lower jaws. Lower jaws much stouter than upper jaws; upper jaws not contacting hyomandibulae; lower jaws thick posteriorly. Hyomandibulae articulate with lower jaws for more than two-thirds of their length (both jaws and one hyomandibula highly distorted in lectotype). At least two pairs of labial cartilages present (one on each set of jaws).

Synarcual cartilage almost as long as neurocranial distance between nasal capsules and occipital segment; synarcual with well developed lateral stays, situated at approximately one-half length of synarcual; lateral stays with slanted anterior and straight posterior margins, and about as wide as suprascapula; four and one-half to five and one-half vertebrae embedded in synarcual anterior to suprascapula.

Propterygium subdivided into five segments, the posterior segment being the longest and close to twice the length of the next longest segment; propterygium articulating with small procondyle of scapulocoracoid; meso- and metapterygium not visible in radiographs and possibly not present, even though very faint radial elements that would be contacting them are clearly visible (meristics are given in table II). Meso- and metacondyles are apparently lacking from scapulocoracoid of lectotype. Propterygium apparently not articulating with antorbital cartilages anteriorly, reaching anteriorly to approximately one-half disc length only. Scapulocoracoid semi-circular in dorsal view, contrasting with suprascapula that is posteriorly elongated. Scapular process laterally stout, contrasting with relatively thin ventral coracoid bar, and dorsally triangular in dorsal view where it articulates with suprascapular bar. Suprascapula appears as a complete bar, extending to just posterior to synarcual cartilage. Fifth ceratobranchials articulate with frontal aspect of scapulocoracoid.

Pelvic girdle with long and thin lateral pre-pelvic processes, reaching close to posterior tips of propterygium; on radiographs, no noticeable flat, expanded areas on distal tips of pre-pelvic processes as in *B. marcida* (these seldomly appear in radiographs); anterior and posterior surfaces of pelvic girdle faintly curved in dorso-ventral



view (surfaces slightly straighter in Somalian specimen); moderately developed, triangular ischiac processes present on posterior surface of pelvic girdle, reaching posteriorly to level of second or third radial element. Stout, slightly curved and not long sub-triangular iliac processes projecting postero-laterally. Radials subdivided only distally (subdivisions not visible in lectotype); first radial greatly enlarged, about twice as thick, and longer than other radials. Basipterygium relatively thin and weakly curved towards mid-line. At least two moderately large obturator foramina present on each side of pelvic girdle; the outer-most foramen is larger. Claspers of adult male (lectotype) with somewhat stout shaft and at least three terminal cartilages, but probably more present; ventral marginal cartilage with thin, pointed segment projecting anteriorly; intermediate clasper segments not visible on radiographs.

Vertebrae less calcified compared to other narcinids (vertebral counts in table II). Vertebral column broken in lectotype at centrum number 12. Anatomy of dorsal and caudal fins not discernible from radiographs as radial elements are weakly calcified.

### Remarks

Although the remaining type-specimens (now paralectotypes) have not been examined, there is little doubt that Alcock's original three specimens are congeneric. However, it cannot be certain that these specimens belong to the same species, even though highly probable, until they can be examined in detail.

*Benthobatis moresbyi* was not illustrated in the original description (Alcock, 1898), but an outline depiction of it appeared shortly thereafter (Alcock, 1899a; Fig. 1 here). This outline clearly illustrates diagnostic features of the species, such as the elongated snout region, almost imperceptible eyes, very long dorsal fin bases, and low, elongated caudal fin. Alcock (1898) mistakenly described *B. moresbyi* as not having lateral tail ridges or folds, and used this as a character to separate *Benthobatis* from other narcinid genera. This has misled subsequent authors who have compiled accounts on the genus, almost always without examining specimens. *B. moresbyi* clearly has a low, albeit inconspicuous, lateral tail ridge. Alcock (1898: 143) believed *Benthobatis* was closely related to *Discopyge*, perhaps on the basis of similarities in their teeth (1898: 144).

The three examined specimens of *B. moresbyi* differ slightly in their relative length of snout (Figs 1-3), although all specimens have a snout longer than in *B. marcida* or *Benthobatis* sp.1. The amount of post-mortem distortion is not precisely known, but seems greater in the specimens from the western Arabian Sea and Indian Ocean, especially BMNH 1909.7.12.7. The snout region, pelvic, dorsal and caudal fins of this specimen are particularly distorted and shrunken compared to the lectotype, but still clearly identify the specimen as *B. moresbyi*. The examined specimens also differ in coloration, as the Somalian specimen has lost most of its original color. The concealed ridges and folded areas of the disc, especially on the ventral snout area, are still dark brown, indicating that the color was originally similar to the other specimens of *B. moresbyi*.

The Somalian specimen (ZMB 17413; Fig. 3) further differs from the other two examined specimens of *B. moresbyi* in the configuration of its nostrils, greater lateral expansion of pelvic fins, slightly less elongated snout (in part due to preservation), stouter antorbital cartilages, and in tooth morphology. The nostrils of this specimen have posteriorly directed processes originating from its anterior margins (Fig. 3C). These projections reach close to one-half in nostril length, and were not observed on the lectotype, but a small process originating from the anterior margins of the nostrils seems to be present in BMNH 1909.7.12.7. However, as the nostrils are highly distorted from

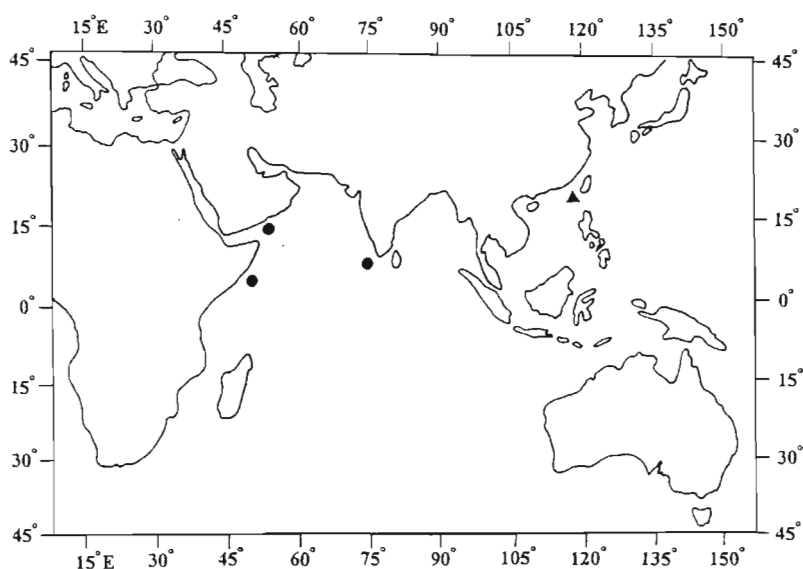


Fig. 5. - Map showing distribution of Indo-Pacific species of *Benthobatis*. All localities are shown. ●: *Benthobatis moresbyi*. ▲: *Benthobatis* sp.2. Type-locality for *B. moresbyi* is continental slope off southwestern coast of India.

their original shape, it is not known whether this represents the same condition as for the Somalian specimen. Compared to the lectotype, the teeth in ZMB 17413 have relatively longer cusps that overlap the bases of teeth situated in the adjacent, inner row. Also the teeth seem more closely packed together. Brauer (1908) considered this specimen as indistinct from the type-specimens of *B. moresbyi*, which he did not examine. Given the few specimens available, this specimen is best identified as *B. moresbyi*, with which it agrees in many features of external and internal morphology, including meristics (Table II). More specimens from northeastern Africa are needed for further comparisons.

Little is known about the anatomy of *B. moresbyi*, as none of the five known specimens were available for dissection. The description of its skeleton given above is based on radiographs of three specimens, including the lectotype, but does not allow for a thorough comparison with other species of *Benthobatis*, especially *B. marcida*, for which cleared and stained and material for dissection is available. However, *B. moresbyi* seems similar to *B. marcida* and both undescribed species in several aspects (e.g., the degree of antero-lateral expansion of the rostrum), suggesting that the genus is monophyletic.

There is no information concerning the general biology or reproduction of this species as well. Of the five known specimens, three are male, one is female and one is an immature specimen (not examined, deposited in the ZSI, Calcutta, referred to only as « young » by Alcock (1898, 1899b, 1899c). Two of the males are adults as judged from the firmness of the claspers of the lectotype (the other adult male, deposited in the ZSI, was not examined, but its total length was given as 14 inches, roughly equivalent to the examined lectotype). The examined immature male is only 120 mm in TL, so maturity is reached between 120 and 350 mm TL. The female specimen examined (ZMB 17413) is presumably adult judging from its size.

### Etymology

*moresbyi*, a patronym named by Alcock « in memory of Capt. Moresby, of the Indian Navy, whose surveys (1834-1838) in the seas where this curious fish is found are known to all readers of Darwin's "Coral-Reefs" (1898: 145).

### Distribution

Known from five specimens collected from both sides of the Arabian Sea and western Indian Ocean area (Fig. 5). All specimens of the type-series were collected together, and the specimen from off Somalia (ZMB 17413) was the fourth collected, even though it was officially reported after Lloyd's (1907) account of the fifth collected specimen (Brauer, 1908). Depths of capture range from approximately 787 to 1071 m.

### Condition of lectotype

The lectotype (BMNH 1898.7.13.22; Fig. 2A, B) is in poor condition, having been dissected on its right dorsal surface from mid-snout area to posterior disc margin. A long ventral slit is also present. A small degree of deformity due to preservation is also noticeable (slight distortion of proportions, expanded cloaca, brittle tips of dorsal and caudal fins), and the mouth is excessively protruded (Fig. 1B). Condition of paralectotypes unknown.

### Referred specimens

BMNH 1909.7.12.7 (Fig. 2C, D), off Yemen, western Arabian Sea (15°55'30"N, 52°38'30"E), 1071 m, 120 mm TL immature male, sta. 358, collected in 1906 by R.I.M.S. "Investigator", bottom temperature approx. 8.6°C, bottom composed of green sandy mud according to Lloyd (1907) (ex-ZSI 1315/1); ZMB 17413 (Fig. 3), off Somalia, western Indian Ocean (4°41'9"N, 48°38'9"E), 823 m, 390 mm TL female, collected 29 Mar. 1899 by the R.V. "Valdivia".

## **BENTHOBATIS MARCIDA BEAN & WEED**

Figs 4B, 6, 7, 9

*Benthobatis marcida* Bean & Weed, 1909: 677 (original description, illustrated; eastern Florida); Garman, 1913: 294-296 (compilation); Chace, 1940 (Cuba); Bigelow and Schroeder 1953: 126-132 (description; South Carolina); Daiber, 1959: 74 (North Carolina); Springer and Bullis, 1956: 43 (listed; Cuba); Bullis and Thompson, 1965: 19 (listed; Florida, Cuba); Miyake, 1988 (anatomical descriptions; numerous pages); Clark and Kristof, 1990: 282 (Bahamas); Eschmeyer *et al.*, 1998: 1015 (listed, recognized as valid); Eschmeyer, 1998: 2182 (listed in a classification).

*Benthobatis cervina* Bean & Weed, 1909: 3 (original description, not illustrated; eastern Florida); Garman, 1913: 294-296 (compilation); Bigelow and Schroeder, 1953: 127-132 (placed in synonymy of *B. marcida*); Eschmeyer *et al.*, 1998: 354 (listed); Eschmeyer, 1998: 2182 (listed in a classification).

*Type-specimens.* - USNM 62916 (holotype of *B. marcida*), off eastern coast of Florida (28°40'N, 78°46'W), 922 m, 485 mm TL, adult female, R.V. "Albatross", sta. 2660, 03 May 1886; USNM 62917 (holotype of *B. cervina*), off eastern coast of Florida (29°41'N, 79°55'W), 683 m, approximately 285 mm TL, adult female, R.V. "Albatross", sta. 2664, 04 May 1886.

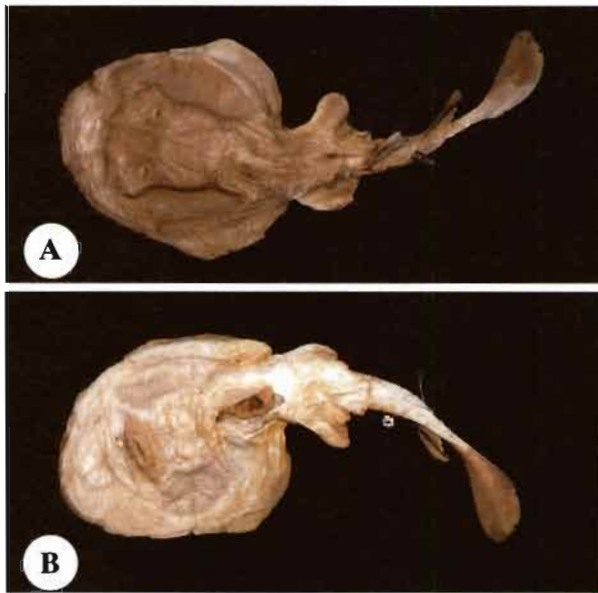


Fig. 6. - Holotype of *Benthobatis marcida* (USNM 62916, female 485 mm TL). A: dorsal view. B: ventral view.

### Diagnosis

A western north Atlantic species distinguished from congeners by having a light brown to yellowish-brown dorsal color pattern; white to yellowish-white ventral color pattern, sometimes with distinct yellow markings on posterior ventral disc surface, ventral pelvic fin area, and around mouth; caudal fin length much less than one-half length of tail, roughly equal to one-fifth length of tail; caudal fin with relatively more pronounced upper and lower lobes, lower lobe strongly convex; distance between second dorsal and caudal fins about equal to distance between first and second dorsal fins; snout relatively short, generally less than one-third in disc length (24% of disc length in 435 mm TL female, 29% in 485 mm TL female), but never much greater than one-third as in *B. moresbyi* (about 40% in lectotype); disc more rounded, not as elongated as in *B. moresbyi* and *Benthobatis* sp.2.

### Remarks

Bean and Weed (1909) did not give precise locality data for the holotype of *B. marcida*. Their only reference is to « Albatross sta. 2660 » which according to Bigelow and Schroeder (1953: 128) corresponds to the coordinates given above. This is close to the type locality of *B. cervina*, which was specified by Bean and Weed (1909). The holotype of *B. cervina* is in poor condition (Fig. 7), and reliable measurements are not possible. However, I confirm that *B. cervina* is a junior synonym of *B. marcida*, following Bigelow and Schroeder (1953). Both type specimens are similar in general proportions (even though these cannot be precisely measured), dorsal and ventral coloration, and shape and position of dorsal and caudal fins. The only characteristic given by Bean and Weed (1909) to separate both species is the relative size of the eyes: *B. cervina* was con-

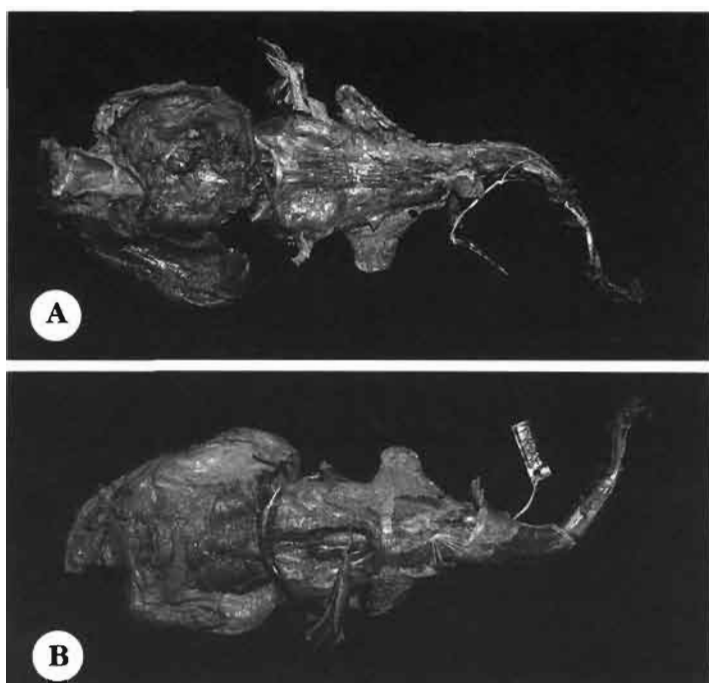


Fig. 7. - Holotype of *Benthobatis cervina* (USNM 62917, female approximately 285 mm TL). A: dorsal view. B: ventral view.

sidered to have "less reduced" eyes compared to *B. marcida*, but this cannot be substantiated from examination of the types or large series of specimens from throughout its range.

One examined specimen (AMNH 56011) is distinct from typical specimens of *B. marcida* because it has more caudal centra (some 52 compared to an average of 31). This specimen (from Florida) also has a correspondingly longer tail, with dorsal fins farther apart. Presently it is identified as *B. marcida* pending further comparisons.

The only available information concerning the reproductive biology of this species is given by Daiber (1959), who reported that a 435 mm TL female had both ovaries functional, approximately 20 mm in diameter, with numerous small eggs of about 2 mm each. Both uteri were functional as each contained one large egg mass (probably composed of fused individual eggs), and the uterine walls were thin and transparent. This resembles the general condition found in many electric rays. Bigelow and Schroeder (1953) report length at birth at being less than 81-87 mm TL.

Specimens observed *in situ* prior to being collected were reported by Clark and Kristof (1990) from off the island of Grand Bahama (3.5 miles south of Freeport). The three specimens observed (two were collected) were reported to be lying on the sand at a depth of 524 m. This constitutes the only observation of *Benthobatis* specimens in their natural habitat.

#### Condition of type-specimens

The holotype of *B. marcida* (Fig. 6) is slightly distorted from its original shape. The disc, pelvic, dorsal and caudal fins are shrunken as if from dehydration, and the colora-

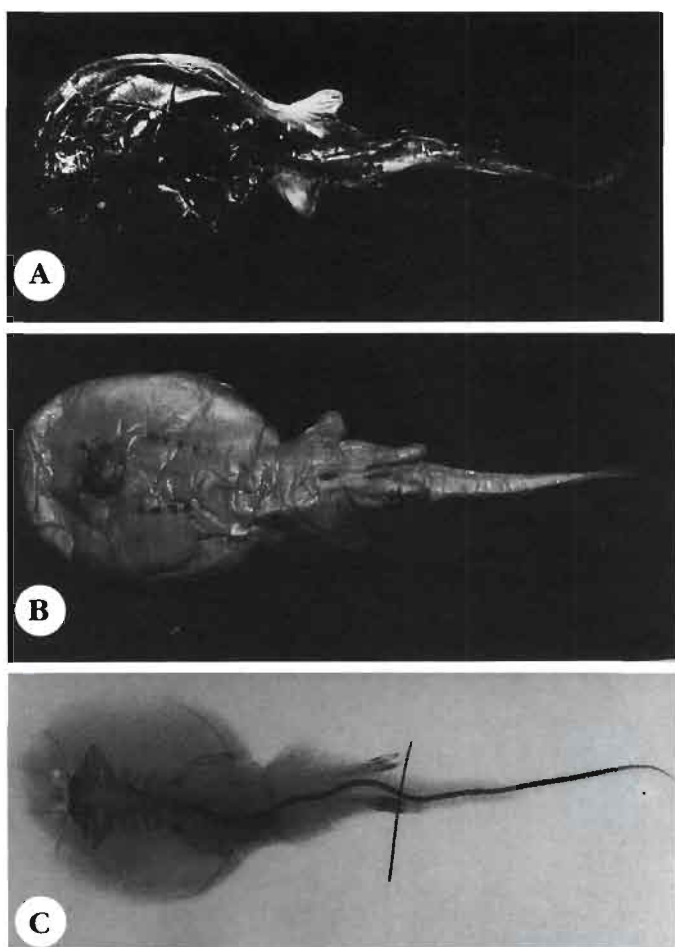


Fig. 8. - *Benthobatis* sp.1 (ZMH-ISH 1989-1968, male 192 mm TL), from off the continental slope of Paraná state, Brazil. A: dorsal view. B: ventral view. C: radiograph showing developed claspers indicative of sexual maturity.

tion is slightly faded. The holotype of *B. cervina* (Fig. 7) is in much worse shape, having decomposed to the point where just touching it will cause it to dismantle further. It had to be "assembled" in order to be photographed and examined. However, certain anatomical features are easily observed, such as the precerebral fontanelle, pattern of the superficial ventral gill arch muscles and eyes.

### Etymology

From the Latin *marcidus*, meaning withered or wasted, in reference to its loose skin and soft aspect, « lacking substance » (Bean and Weed, 1909: 679).

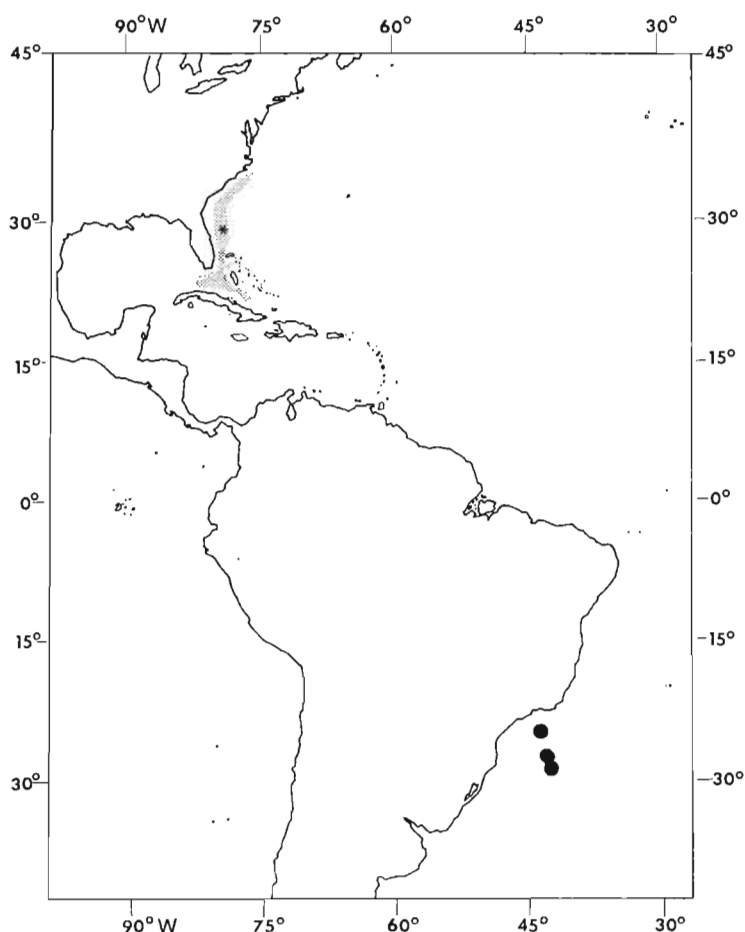


Fig. 9. - Map showing known distribution of Atlantic species of *Benthobatis*. Shaded region in north Atlantic is *B. marcida* (asterisk indicates type-locality). ● is *Benthobatis* sp.1 (all localities shown for this species).

### Distribution

An offshore, continental slope species, apparently abundant in certain localities, known from off southern North Carolina (Daiber, 1959), South Carolina (Bigelow and Schroeder, 1953; Daiber, 1959), Georgia (USNM 37886), eastern and southern Florida (Bean and Weed, 1909; Bullis and Thompson, 1965), the Bahamas (Clark and Kristof, 1990; USNM 197142) and northern and northwestern continental slope of Cuba (Chace, 1940; Bigelow and Schroeder, 1953; Springer and Bullis, 1956; Bullis and Thompson, 1965) (Fig. 9). Possesses a great bathymetric distribution, occurring in depths of approximately 274 to 923 m.

### Referred specimens

(More data available on request; not all lots examined.) AMNH 56011 (Florida); FMNH 66138 (Cuba); FMNH 65734 (Cuba); MCZ 36984 (Cuba); MCZ 36999 (Florida);

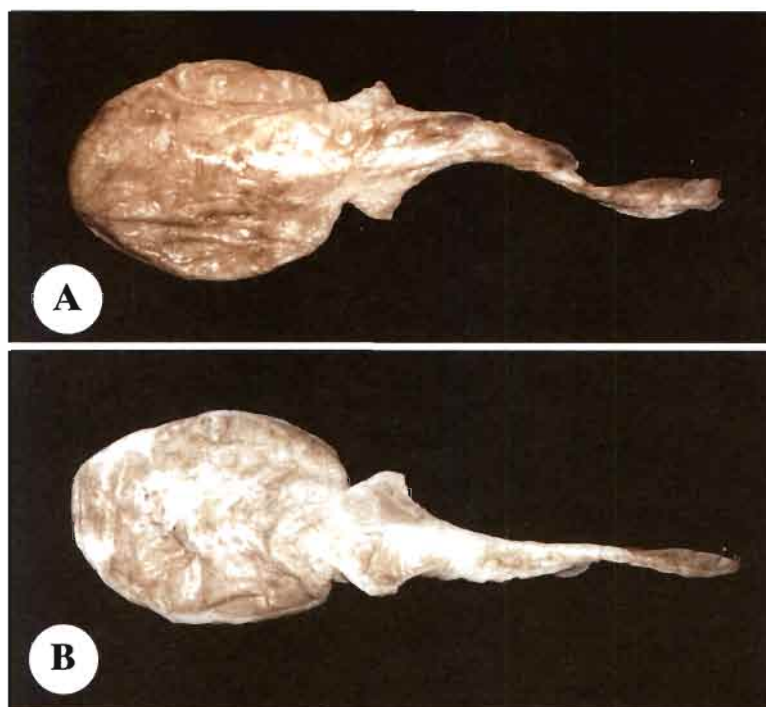


Fig. 10. - *Benthobatis* sp.2 (NTUM 01712, female 257 mm TL), from the southwestern coast of Taiwan. A: dorsal view. B: ventral view.

MCZ 37108 (Cuba); MCZ 37109 (Cuba); MCZ 37110 (Cuba); MCZ 37111 (Cuba); MCZ 37112 (Cuba); MCZ 37113 (Cuba); MCZ 37114 (Cuba); MCZ 37115 (Cuba); MCZ 37116 (Cuba); MCZ 37117 (Cuba); MCZ 37118 (Cuba); MCZ 37119 (Cuba); MCZ 37120 (Cuba); MCZ 37121 (Cuba); MCZ 37122 (Cuba); MCZ 37123 (Cuba); MCZ 37124 (Cuba); MCZ 37125 (Cuba); MCZ 37126 (Cuba); MCZ 37127 (Cuba); MCZ 37128 (Cuba); MCZ 37129 (Cuba); MCZ 37130 (Cuba); MCZ 37131 (Cuba); MCZ 37132 (Cuba); MCZ 37133 (Cuba); MCZ 37134 (Cuba); MCZ 39920 (Florida); MCZ 39921 (Florida); MCZ 40095 (Bahamas); MCZ 40103 (Straits of Florida); MCZ 40133 (Straits of Florida); MCZ 41117 (Cuba); MCZ 41161 (Cuba); MCZ 41169 (Cuba); MCZ 41171 (Cuba); MCZ 41831 (Florida); MCZ 41932 (Cuba); MCZ 41979 (Cuba); MCZ 41987 (Bahamas); MCZ 41989 (Straits of Florida); MCZ 42001 (Bahamas); MCZ 42005 (Straits of Florida); MCZ 48989 (Florida); USNM 37886 (Georgia); USNM 76582 (South Carolina); USNM 157984 (Grand Bahama Is.); USNM 186428 (Cuba); USNM 197114 (Florida); USNM 197142 (Grand Bahama Is.); USNM 222426 (Cuba); USNM 222429 (Cuba).



**BENTHOBATIS SP. 1**

Figs 8, 9

**Diagnosis**

A small species of *Benthobatis* distinguished from *B. marcida* by its much darker dorsal color pattern (dark, purplish-brown compared to light brown in *B. marcida*); pelvic fins originating posterior to disc (disc slightly overlapping pelvics in well preserved specimens of *B. marcida*); first dorsal fin originating over posterior tips of pelvic fins (originating anterior to posterior tips of pelvics in *B. marcida*); dorsal fins low, with bases about three-fourths in dorsal fin height; caudal fin very low and relatively long; smaller size at maturity (examined mature male of 192 mm TL with well developed claspers [Fig. 8C], compared to males of *B. marcida* which still have rudimentary claspers at 220 mm TL). *Benthobatis* sp.1 can be separated from *B. moresbyi* by its white to yellowish-white ventral color pattern, shorter snout length, and position and shape of dorsal and caudal fins (dorsal fins not longer than tall, and not as stout and close together as those of *B. moresbyi*; in *Benthobatis* sp.1 second dorsal fin is closer to caudal fin than to first dorsal fin, and the first dorsal fin is positioned further back on the body, posterior to pelvic fins).

**Remarks**

Only recently reported, this species may be the smallest of the four known species of *Benthobatis*. The original report<sup>(4)</sup> was based on specimens collected in three sets of otter trawls in September and October of 1991. The first collected specimens, however, date from 1967 and were collected by the "Walther Herwig" from the continental slope off the coast of Paraná state (Brazil). This species is locally abundant as 148 specimens were sampled together from the three localities of 1991. A female of 233 mm TL and a male of 180 mm TL are reported to be adults. *Benthobatis* specimens may be present in the Museo Argentino de Ciencias Naturales "Bernadino Rivadavia" (G. Chiaramonte, pers. comm.), but this remains unconfirmed.

**Distribution**

Known only from the southwestern Atlantic (Fig. 9) from four distinct localities (ranging from 26°30'S to 29°47.63'S and 46°11'W to 47°48.01'W), off the continental slope of the Brazilian states of Paraná, Santa Catarina and Rio Grande do Sul, from depths of 470 to 524 m.

**Referred specimens**

ISH-ZMH 1989-1968, off southwestern Brazil, Paraná state (26°30'S, 46°11'W), 485 m, collected by the F.F.S. "Walther Herwig", sta. 116-68, 12 Mar. 1967.

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(4) C. Vooren and G. Rincón, abstract (see footnote 1). Locality data and sexual maturity for females given in this paragraph is largely extracted from their abstract. This species is presently being described by these authors along with M. Stehmann.

**BENTHOBATIS SP.2**

Figs 4C, 5, 10

*Benthobatis moresbyi*: Chen and Chung, 1971: 5-6, fig. 3 (misidentification, description, illustration); Shen, 1984a: 5, pl. 5 (misidentification; brief description, photograph; southwestern Taiwan); Shen, 1984b: 40 (name only; misidentification); Yu, 1988: 9 (listed); Chen and Joung, 1993: 79, 621, pl. 11 (misidentification; brief description, photograph; southwestern Taiwan).

*Benthobatis* sp.: Carvalho *et al.*, in press (identification, brief description).

**Diagnosis**

Distinguished from other species of *Benthobatis* by the combination of: dark brown to blackish-brown dorsal and ventral coloration, marked with small areas of lighter pigment lateral to ventral snout region, anterior to pelvic fins dorsally and ventrally, under tail posterior to pelvic fins, and on sides of tail and pelvic fin area; mouth width approximately one-ninth to one-tenth in disc width, compared to one-seventh in *B. moresbyi*; first dorsal fin originates over posterior one-third of pelvic fins; disc not overlapping pelvic fin origins; pelvic fins without prominent lateral lobe anteriorly; first dorsal fin triangular in outline, with straight anterior margin at more or less 45° angle to tail, and posterior margin contacting tail without leaving free lobe; second dorsal fin more rounded in outline compared to first dorsal fin, with highly curved anterior margin, rounded upper aspect, and convex posterior margin leaving slight free lobe posteriorly; dorsal fins with length slightly greater than height (bases close to twice as long as high in *B. moresbyi*); second dorsal fin slightly closer to caudal fin than to first dorsal fin; suprascapula very straight across vertebral column, not curved posteriorly as in other species of *Benthobatis*; pelvic girdle with distinct posteriorly directed triangular processes on posterior margin, mesial to ischiac processes; greater number of caudal fin vertebrae (43 compared to 38 in lectotype of *B. moresbyi*); greater number of caudal fin ventral radial elements (40 compared to some 26 in lectotype of *B. moresbyi*).

**Remarks**

Chen and Chung (1971) provide the first occurrence of *Benthobatis* from the Pacific ocean. Their material consisted of 15 specimens ranging from 135 to 316 mm in total length, however they do not state if both males and females were present in their sample. The material apparently was part of the ichthyological collection of Tunghai University (Taichung, Taiwan), but no further details concerning their capture or locality are given in their account. One specimen, perhaps a female, was illustrated by the junior author, providing indications that their specimens differed from *B. moresbyi* at least in proportions of the dorsal and caudal fins. Their description clearly states that a low lateral tail fold or ridge is present, along with rudimentary but visible eyes, 10 rows of teeth, and a purplish-black dorsal coloration with scattered white pores on disc and white blotches on tips of second dorsal and caudal fins. However, their method of counting teeth is not described, prohibiting further comparisons.

Shen (1984a) and Chen and Joung (1993) recorded and figured two different specimens of this undescribed species. The specimen in figure 10 is the one shown in Shen (1984a). A female specimen of about 171 mm TL, similar to the one in figure 10 and also from southwestern Taiwan, was collected from depths of probably less than 300 m (L.J.V. Compagno, pers. comm.). Three specimens from the same area were found in the

collections of the Scripps Institute of Oceanography (165 to 215 mm TL, all males). On the basis of these three specimens, sexual maturity for males must occur at around 195 mm TL. Twenty specimens have been recorded so far (at least six of which are extant in collections), and the four examined specimens are similar in external morphology and coloration. This species is presently being described<sup>(5)</sup>.

*Benthobatis* sp.2 is most similar to *B. moresbyi*. Both species have dark brown ventral coloration patterns, elongated snouts (preorbital distance more than one-third in disc length), long caudal fins (one-half or more length of tail as measured from posterior margins of pelvic fins), and dorsal fins with long, fleshy bases (base length greater than dorsal fin height). These species differ in counts of vertebrae, ventral caudal fin radial elements, and in relative size and position of dorsal fins, as *Benthobatis* sp.2 has proportionally smaller dorsal fins and a much longer interdorsal distance, as well as a greater distance between second dorsal and caudal fins. *Benthobatis* sp.2 further differs from the Somalian specimen of *B. moresbyi*, and to some degree from the lectotype, in having cusps that do not overlap teeth in adjacent inner rows, and teeth with wider bases and shorter cusps.

### Distribution

Collected only from southwestern Taiwan (Fig. 5). Precise bathymetric distribution unknown, but one female specimen of 171 mm TL, collected by a shrimp trawler, is believed to be from less than 300 m (found in the fish market of Tung-Kang, Taiwan; L.J.V. Compagno, pers. comm.).

### Referred specimens

NTUM 01712, 257 mm TL, female, off southwestern Taiwan, « 1972, 11, 13 » (no further information available); SIO 70-274, 3 male specimens, 165 to 215 mm TL, Tung-Kang, Taiwan, collected by L. Chen, 30 Jun 1970.

**Acknowledgements.** - For loans of specimens, technical help and hospitality during museum visits, I thank Anthony C. Gill, Darrell Siebert, Oliver Crimmen, Sean Davidson and Patrick Campbell (NHM); Hans-Joachim Paepke (ZMB); Horst Wilkens, Matthias Stehmann and Gudrun Schultze (ZMH); Karsten Hartel and Karel Liem (MCZ); Susan L. Jewett, Jeffrey T. Williams, Lynne R. Parenti, Jerome Finnan, Lisa Palmer and Sandra Raredon (USNM); John D. McEachran (Texas A & M University); Shih-chieh Shen and Wen-Bin Huang (NTUM); H.J. Walker Jr. (SIO). Scott A. Schaefer (AMNH) provided computer assistance. Darrell Siebert provided slides of the lectotype of *B. moresbyi* and Craig Chesek (AMNH) provided assistance with photography. For much useful discussion or information concerning this paper or related issues, I thank Valerie Schawaroch and Ian Harrison (AMNH), Leonard J.V. Compagno (SAM), Peter Last (CSIRO), Monica Toledo-Piza (MZUSP), H.-J. Paepke, John D. McEachran, Anthony C. Gill and an anonymous reviewer. Support from the Department of Ichthyology and Office of Grants and Fellowships (both AMNH) is gratefully appreciated. I would also like to thank the organizers of the 5th Indo-Pacific Fishes meetings for logistic support, especially Michel Kulbicki and Bernard Séret. For hospitality, friendship and support in Europe, thanks are due to Helga Friedheim (Hamburg) and Molly, Nat and Tony Gill (London).

This work was supported in part by grants from the Lerner-Gray Fund for Marine Research (AMNH), the Donn Rosen Fund of the Department of Ichthyology (AMNH), a Doctoral Fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) of the Brazilian Federal government, a Research Grant from Friends of Fishes, Inc. (New York), the Dissertation Grant

(5) L.J.V. Compagno, M.R. de Carvalho and D. Ebert, manuscript in preparation.

Program of The City University of New York, and a Doctoral Fellowship from the American Museum of Natural History.

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## ELASMOBRANCH BIODIVERSITY, CONSERVATION AND MANAGEMENT IN SABAH (MALAYSIA)

by

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**ABSTRACT.** - The first detailed study of shark and ray (elasmobranch) biodiversity in Sabah, East Malaysia is presented. A large number of species (34 sharks and 35 rays) were identified during fish market visits and field surveys, 23 of which were new records for Sabah, including an undescribed river shark *Glyphis* sp. Several other species, some of which are probably also undescribed, could only be identified to genus level. New records were made throughout the study period, and the species list is certainly still incomplete. Information was also gathered on the geographical and seasonal distribution and abundance of several elasmobranchs. Of particular interest were the new records of elasmobranchs from some of the largest rivers, representing just some of the freshwater species confidently described by local fishermen. A reference collection is now available in Sabah to support future taxonomic studies and related research work. The project also 1) examined the socio-economic importance of elasmobranchs in subsistence and commercial fisheries; 2) provided educational materials to increase the local community's awareness of sharks and rays; 3) considered problems and opportunities for conservation and management (particularly concerning fisheries and habitat issues); and 4) concluded with an international workshop to develop recommendations for the conservation and management of elasmobranch populations and their critical habitats. The final workshop was also used to disseminate more widely both the results of the Sabah project and that of other elasmobranch research underway in the region. These data should provide the information needed by decision-makers to begin to advance elasmobranch conservation and management in Sabah and Malaysia, provide a sound basis for future taxonomic and ecological research in the region, and stimulate future studies in Sabah and other Malaysian States and nearby countries.

**RÉSUMÉ.** - Biodiversité, conservation et gestion des élamobranthes au Sabah (Malaisie).

La première étude détaillée sur la biodiversité des requins et des raies (élamobranthes) au Sabah (Malaisie orientale) est présentée. Un nombre élevé d'espèces (34 requins et 35 raies) ont été identifiées durant les missions sur le terrain et sur les marchés locaux, et 23 d'entre elles sont des nouveaux signalements pour le Sabah, dont une nouvelle espèce de requin du genre *Glyphis*. Plusieurs autres espèces, certaines probablement nouvelles, n'ont pu être identifiées qu'au niveau du genre. Tout au long de l'étude, de nouveaux signalements ont été notés, si bien que la liste actuelle est certainement

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incomplète. Des données sur les répartitions géographique et saisonnière, et l'abondance de plusieurs espèces d'élasmobranches, ont également été collectées. Les nouveaux signalements d'élasmobranches dans les grandes rivières sont particulièrement remarquables; ils représentent seulement quelques-unes des espèces qui ont été mentionnées par les pêcheurs locaux. Une collection de référence est maintenant disponible au Sabah pour des études taxinomiques et d'autres recherches. Le projet 1) a évalué l'importance socio-économique des élasmobranches dans les pêcheries de subsistance et commerciales; 2) a fourni du matériel éducatif pour sensibiliser davantage les communautés locales aux problèmes des requins et des raies; 3) a déterminé les problèmes et les opportunités de conservation et de gestion (concernant notamment les pêcheries et les habitats); et 4) s'est achevé par la tenue d'un atelier international qui a proposé des recommandations pour la conservation et la gestion des populations d'élasmobranches et de leurs habitats menacés. L'atelier a aussi permis de diffuser plus largement les résultats du projet et ceux d'autres recherches sur les élasmobranches en cours dans la région. Ces données devraient fournir aux décideurs l'information nécessaire pour assurer la conservation et la gestion des élasmobranches du Sabah et de Malaisie, et disposer d'une base solide pour entreprendre des recherches taxinomiques et écologiques, et pour induire d'autres recherches au Sabah et dans les autres états de Malaisie, ainsi que dans les pays limitrophes.

Key-words. - Elasmobranchii, Malaysia, Sabah, Biodiversity, Conservation, Freshwater, Management, Market surveys.

The conservation problems faced by freshwater and marine elasmobranchs in Southeast Asia were the subject of discussion by IUCN Shark Specialist Group members during and after the 4<sup>th</sup> International Indo-Pacific Fish Conference in Bangkok, Thailand, 1994. Although there were no records of freshwater elasmobranchs in published literature for Sabah, East Malaysia, (Inger and Chin, 1962; Lim and Wong, 1994), they are recorded elsewhere in Borneo (e.g., Roberts, 1989). In 1995, the senior author spent a few days on the largest river in Sabah, the Kinabatangan, where Orang Sungei (river people) fishermen reported that they fairly regularly caught at least two species of ray over 200 km from the sea, and very rarely sharks and sawfishes (Fowler and Payne, 1995). There was also a diverse range of sharks and rays on sale in the larger fish markets of Sabah, despite reports of falling elasmobranch catches and biodiversity levels elsewhere in Southeast Asia. Further study of this area appeared to be timely. The Shark Specialist Group applied for grant-aid from the UK Darwin Project for the Survival of Species for an 18 month study of the biodiversity, conservation and management of sharks and rays in Sabah.

The project was carried out from early 1996 to mid 1997, in collaboration with the Department of Fisheries (DoF) Sabah and with assistance from WWF Malaysia. It aimed to undertake the first dedicated study on the biodiversity of elasmobranchs (sharks and rays) in the region; to examine their socio-economic importance in commercial fisheries and for subsistence communities; to consider the problems and opportunities for conservation

Log 3 Abundance category	Number of fishes
1	1
2	2-3
3	4-9
4	10-27
5	28-81

Table I. - Logarithmic abundance categories used to estimate the abundance of numerically-dominant species in fish markets in Sabah, East Malaysia.



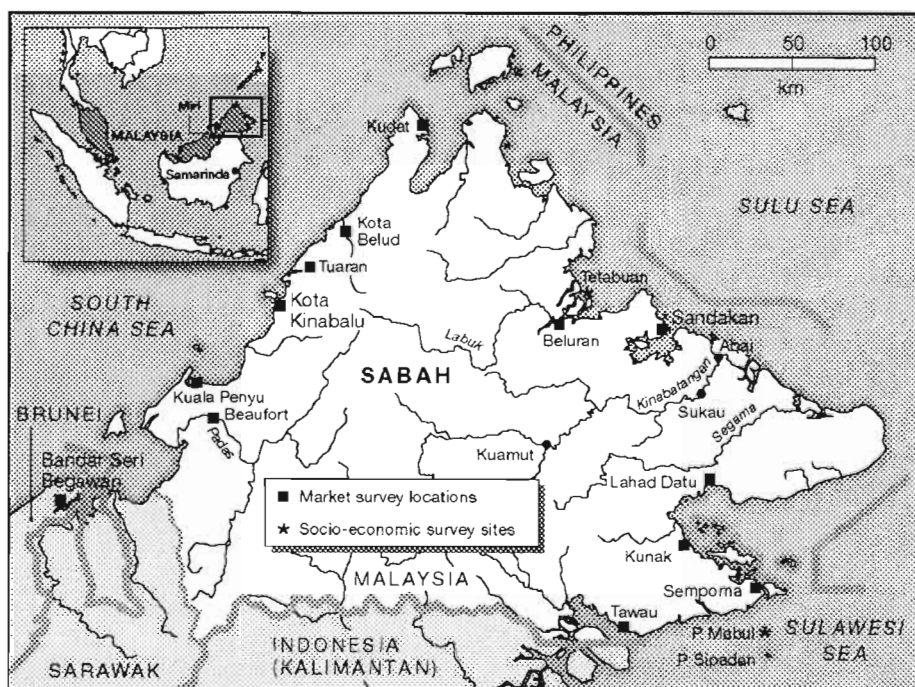


Fig. 1. - Location of main study areas in Sabah, East Malaysia.

and management (particularly fisheries and habitat issues); to provide educational materials for local communities and increase their awareness of sharks and rays; and to develop recommendations for the management of shark and ray populations and their critical habitats. It was intended that data obtained during the study would begin to provide the information required by decision-makers to advance elasmobranch conservation in Sabah and Malaysia.

## MATERIAL AND METHODS

The Darwin project team consisted of a part time UK-based Project Leader (the senior author), a full time Sabahan Project Officer (the second author), a few long term volunteers (usually based in Sabah for two month periods), and occasional visiting experts from the Shark Specialist Group. The local team was based in Kota Kinabalu, where the DoF provided accommodation and office facilities. The Department also supplied additional vehicles, boats and support staff for some field surveys and nominated a Fishery Officer for project liaison. The project grant covered travel and subsistence expenses during fieldwork, and the purchase of a small project vehicle and other minor capital equipment and consumables, including temporary containers for the large reference collection.

The study was mainly carried out by a combination of regular fish market visits, river surveys, and visits to river and coastal kampungs (villages) where villagers could be interviewed. Additional information was obtained from DoF records and experienced Fisheries and WWF staff.

### **Biodiversity study**

A programme of regular visits to all the main fish markets in Sabah was undertaken, supplemented by occasional visits to markets in neighbouring states and countries in Borneo (Fig. 1). A checklist of elasmobranchs was produced (Table II), supported where possible by specimens collected for the reference collection, or (for larger fish) by photographs and written records. Species identification was mainly carried out with Compagno (1984) and Last and Stevens (1996). The study was hampered by the lack of a key to batoids for the region, and much of the species identification work would not have been possible without the expertise of visiting taxonomists. Single use cameras were issued to several villagers and Fisheries Officers so that unusual landings could be recorded, and additional information was obtained from photographs sent in to the project team. Initial proposals to carry out diving surveys were abandoned because this methodology was considered expensive and unlikely to yield more than a few records of relatively common species. Records were also made during DoF surveys and occasional trips on fishing vessels.

Towards the end of the project, market survey methodology was refined to include rapid log<sub>3</sub> estimates of abundance (see Table I). This helped to provide a semi-quantified indication of variations in seasonal abundance for some species, and comparisons of the abundance of different species. Tissue samples were also collected for genetic analysis.

The survey team carried out regular freshwater sampling using long lines and gill nets in the Kinabatangan and Segama rivers and oxbow lakes. Methodology is described in more detail by Manjaji (in press, a). The project team's elasmobranch sampling efforts were generally unsuccessful, largely due to high river levels and the rarity of the target species, which are only caught infrequently by full time fishermen. However, an agreement was reached with some fishermen along the Kinabatangan that they would save specimens of freshwater sharks and rays taken as bycatch (they were asked not to target these rare species for the project) using the same fishing methods. Three families were supplied with lockable poly-ethylene tanks filled with 70% formalin, in which to preserve any small specimens caught, and single use cameras were left with key individuals so that photographs could be taken of larger fish landed. The current market price was paid for all elasmobranchs kept.

### **Socio-economic study**

Two kampongs in eastern Sabah, Kg. Pulau Tetabuan (on an estuary) and Kg. Pulau Mabul (on a reef island), were studied during a two week pilot socio-economic study. Interviews were carried out in both villages to gather information on fish species, catches, prices, and human nutrition. Data gathered were entered in a survey form based on methodology developed for similar projects in Asia and Africa (Almada-Villela in press). Villagers were also questioned about traditional uses of sharks and rays.

### **Education and training for local people**

This was an important component of the Darwin project. Informal training was provided to the Sabahan Project Officer by visiting experts throughout the project. Other DoF staff and volunteers also benefited in this way. Discussions and interviews with local fishermen and villagers, including village heads and elders, enabled two-way flow of information and ideas and provided opportunities for the team to discuss fisheries and conservation issues with community leaders and obtain their support for the project while acquiring information on past and present elasmobranch catches. Finally, the Darwin

Table II. - Checklist of elasmobranchs recorded from Sabah and elsewhere in Borneo. (1). Recorded from Sabah during project survey period (January 1996-June 1997); (2). First record from Sabah; (3). Recorded elsewhere in Borneo, not yet recorded from Sabah (Cook and Compagno, 1996); (4). Miri, northern Sarawak (July 1996) (p: photo only); (5). Samarinda, east Kalimantan (November 1996) (p: photo only; s: specimen saved); (6). Bandar Seri Begawan, Brunei (January 1997) (s: specimen saved).

SHARKS	1	2	3	4	5	6
<b>Order Carcharhiniformes (Ground sharks)</b>						
<b>Family Carcharhinidae (Requiem sharks)</b>						
<i>Carcharhinus amblyrhynchoides</i> (Whitley, 1934). Graceful shark.	x					
<i>Carcharhinus amblyrhynchos</i> (Bleeker, 1856). Grey reef shark.	x	x				
<i>Carcharhinus borneensis</i> (Bleeker, 1859). Borneo shark.			x			
<i>Carcharhinus brevipinna</i> (Müller & Henle, 1839). Spinner shark.	x					
<i>Carcharhinus dussumieri</i> (Valenciennes, in Müller & Henle, 1839). Whitecheek shark.	x					
<i>Carcharhinus falciformis</i> (Bibron, 1839). Silky shark.	x	x				
<i>Carcharhinus leucas</i> (Valenciennes, in Müller & Henle, 1839). Bull shark.	x					
<i>Carcharhinus limbatus</i> (Valenciennes, in Müller & Henle, 1839). Blacktip shark.	x					
<i>Carcharhinus melanopterus</i> (Quoy & Gaimard, 1824). Blacktip reef shark.	x					
<i>Carcharhinus sealei</i> (Pietschmann, 1916). Blackspot shark.	x					
<i>Carcharhinus sorrah</i> (Valenciennes, in Müller & Henle, 1839). Spot-tail shark.	x					
<i>Carcharhinus</i> sp. [Compagno, 1988]. False smalltail shark.			x			
<i>Carcharhinus plumbeus</i> (Nardo, 1827). Sandbar shark.	x					
<i>Galeocerdo cuvier</i> (Péron & LeSueur, 1822). Tiger shark.	x					
<i>Glyphis</i> sp.B [Compagno, Mycock, Cavanagh, Manjaji & Fowler] Borneo river shark.	x	x				
<i>Lamiopsis temminckii</i> (Müller & Henle, 1839). Broadfin shark.			x			
<i>Loxodon macrorhinus</i> Müller & Henle, 1839. Slit-eye shark.	x	x				
<i>Rhizoprionodon acutus</i> (Rüppell, 1837). Milk shark.	x					
<i>Rhizoprionodon oligolinx</i> Springer, 1964. Grey sharpnose shark.	x	x				
<i>Scoliodon laticaudus</i> Müller & Henle, 1838. Spadenose shark.	x			x		
<i>Triaenodon obesus</i> (Rüppell, 1837). Whitetip reef shark.	x					
<b>Family Hemigaleidae (Weasel sharks)</b>						
<i>Chuenogaleus macrostoma</i> (Bleeker, 1852). Hooktooth shark.	x					
<i>Hemigaleus microstoma</i> Bleeker, 1852. Sickletfin weasel shark.	x					
<i>Hemipristis elongata</i> (Klunzinger, 1871). Snaggletooth shark.	x					
<b>Family Scyliorhinidae (Cat sharks)</b>						
<i>Apristurus sibogae</i> (Weber, 1913). Pale catshark.			x			
<i>Apristurus verweyi</i> (Fowler, 1934). Borneo catshark.			x			
<i>Atelomyxterus marmoratus</i> (Bennett, 1830). Coral catshark.	x	x				
<b>Family Sphyrnidae (Hammerhead sharks)</b>						
<i>Eusphyra blochii</i> (Cuvier, 1817). Winghead shark.	x	x				
<i>Sphyrna lewini</i> (Griffith & Smith, in Cuvier, Griffith & Smith, 1834). Scalloped hammerhead.	x					
<i>Sphyrna mokarran</i> (Rüppell, 1837). Great hammerhead.	x	x				
<b>Family Triakidae (Hound sharks)</b>						
<i>Mustelus</i> sp.1 [Compagno.] Sabah white-spotted smoothhound.	x	x				
<i>Mustelus</i> sp. 2. Grey smoothhound.	x	x				
<b>Order Heterodontiformes (Bullhead sharks)</b>						
<b>Family Heterodontidae (Bullhead sharks)</b>						
<i>Heterodontus zebra</i> (Gray, 1831). Zebra bullhead shark.	x	x				

Table II.- Continued.

SHARKS	1	2	3	4	5	6
<b>Order Orectolobiformes (Carpet Sharks)</b>						
<b>Family Hemiscylliidae (Longtailed carpet sharks)</b>						
<i>Chiloscyllium hasselti</i> Bleeker, 1852. Indonesian bambooshark.			x			
<i>Chiloscyllium plagiosum</i> (Bennett, 1830). Whitespotted bambooshark.	x	x				
<i>Chiloscyllium punctatum</i> Müller & Henle, 1838. Brownbanded bambooshark.	x					
<b>Family Orectolobidae (Wobbegongs)</b>						
<i>Orectolobus</i> sp. [Last, Manjaji, & Fowler]. Borneo wobbegong.	x	x				
<b>Family Rhincodontidae (Whale shark)</b>						
<i>Rhincodon typus</i> Smith, 1829. Whale shark.	x					
<b>Family Stegostomatidae (Zebra shark)</b>						
<i>Stegostoma fasciatum</i> (Hermann, 1783). Zebra shark.	x					
<b>Order Squaliformes (Dogfish sharks)</b>						
<b>Family Squalidae (Dogfish sharks)</b>						
<i>Centrophorus moluccensis</i> Bleeker, 1860. Smallfin gulper shark.	x	x				
<b>BATOIDS (RAYS)</b>						
<b>Order Myliobatiformes (Stingrays)</b>						
<b>Family Dasyatidae (Whiptail stingrays)</b>						
<i>Dasyatis kuhlii</i> (Müller & Henle, 1841). Bluespotted maskray.	x					
<i>Dasyatis microps</i> (Annandale, 1909). Smalleye stingray.	x	x				
<i>Dasyatis zugei</i> (Müller & Henle, 1841). Pale-edged stingray.	x					
<i>Himantura chaophraya</i> Monkolprasit & Roberts, 1990. Giant freshwater stingray.	x	x				
<i>Himantura fai</i> Jordan & Seale, 1906. Pink whipray.	x	x				
<i>Himantura fuva</i> (Annandale, 1909). Honeycomb whipray.	x	x				
<i>Himantura gerrardi</i> (Gray, 1851). Sharpnose stingray/ Whitespot whipray.	x					
<i>Himantura jenkinsii</i> (Annandale, 1909). Jenkins whipray/ Golden whipray.	x					
<i>Himantura pastinacoides</i> (Bleeker, 1852). Round whipray.	x					
<i>Himantura signifer</i> Compagno & Roberts, 1982. White-edged freshwater whipray.			x			
<i>Himantura uarnacoides</i> (Bleeker, 1852). White-nosed whipray.	x					
<i>Himantura uarnak</i> Forsskal, 1775. Reticulate whipray.	x					
<i>Himantura undulata</i> (Bleeker, 1852). Leopard whipray.	x	x				
<i>Himantura walga</i> (Müller & Henle, 1841). Dwarf whipray.	x					
<i>Pastinachus sephen</i> (Forsskal, 1775). Cowtail stingray.	x					
<i>Pastinachus</i> sp. [Last.] Roughnose stingray.	x					x
<i>Taeniura lymna</i> (Forsskal, 1775). Bluespotted fantail ray.	x					
<i>Taeniura meyeni</i> Müller & Henle, 1841. Blotched fantail ray.	x	x				
<i>Urogymnus asperrimus</i> (Bloch & Schneider, 1801). Porcupine ray.	x				p	
<b>Family Gymnuridae (Butterfly rays)</b>						
<i>Aetoplatea zonura</i> Bleeker, 1852. Zonetail butterfly ray.	x					
<i>Gymnura poecilura</i> (Shaw, 1804). Longtail butterfly ray.	x					
<b>Family Mobulidae (Devilrays)</b>						
<i>Manta birostris</i> (Donndorff, 1798). Manta ray.			x			
<i>Mobula eregoodootenkee</i> (Cuvier, 1829). Pygmy devilray.	x					
<i>Mobula japanica</i> (Müller & Henle, 1841). Japanese devilray.	x	x				
<i>Mobula thurstoni</i> (Lloyd, 1908). Bentfin devilray.	x	x				

Table II. - Continued 2.

BATOIDS (RAYS)	1	2	3	4	5	6
<b>Family Myliobatidae (Eaglerays)</b>						
<i>Aetobatus narinari</i> (Euphrasen, 1790). Spotted eagle ray.	x					
<i>Aetomylaeus maculatus</i> (Gray, 1832). Mottled eagle ray.	x					
<i>Aetomylaeus nicholfii</i> (Schneider, 1801). Banded eagle ray.	x					
<i>Aetomylaeus vespertilio</i> (Bleeker, 1852). Ornate eagle ray.	x	x				
<b>Family Rhinopteridae (Cownose rays)</b>						
<i>Rhinoptera javanica</i> Müller & Henle, 1841. Javanese cownose ray.	x					
<b>Order Rajiformes (Skates)</b>						
<b>Family Anacanthobatidae (Legskates)</b>						
<i>Anacanthobatis borneensis</i> Chan, 1965. Borneo legskate.			x			
<b>Order Rhinobatiformes (Guitarfishes)</b>						
<b>Family Rhinobatidae (Guitarfishes)</b>						
<i>Rhinobatos granulatus</i> Cuvier, 1829. Sharpnose guitarfish.			x			
<i>Rhinobatos thoun</i> (Anonymous, 1798). Clubnose guitarfish.					s	
<i>Rhinobatos typus</i> Bennett, 1830. Giant shovelnose ray.	x					
<b>Order Rhiniformes (Sharkfin guitarfishes or wedgefishes)</b>						
<b>Family Rhinidae (Sharkfin guitarfishes or Wedgefishes)</b>						
<i>Rhina ancylostoma</i> Bloch & Schneider, 1801. Sharkray.	x	x				
<i>Rhynchobatus</i> sp. 2 [Compagno & Last-WPLC]. Broadnose wedgefish.	x					
<i>Rhynchobatus australiae</i> Whitley, 1939. White-spotted wedgefish (WPHC).	x					
<b>Order Torpediniformes (Electric rays)</b>						
<b>Family Narkidae (Shortnose electric rays)</b>						
<i>Narke dipterygia</i> (Bloch & Schneider, 1801). Spottail electric ray.			x			
<b>Order Pristiformes (Sawfishes)</b>						
<b>Family Pristidae (Modern sawfishes)</b>						
<i>Anoxypristis cuspidatus</i> (Latham, 1794). Narrow sawfish.	x	x				
<i>Pristis microdon</i> Latham, 1794. Greattooth or freshwater sawfish.	x					
<i>Pristis zijsron</i> Bleeker, 1851. Green sawfish.	x					
<b>CHIMAERAS:</b>						
<b>Order Chimaeriformes (Chimaeras)</b>						
<b>Family Chimaeridae (Shortnose Chimaeridae)</b>						
<i>Chimaera</i> sp. [Didier]. Borneo chimaera.			x			

project funded the production of leaflets and posters about the conservation of Sabah's rivers and the importance of their wildlife, including sharks and rays. These were printed in English and Malay and distributed by the project team and WWF staff to communities and schools along the rivers, to help raise the awareness of local people about these conservation issues.

#### International seminar and workshop

The Darwin Initiative and World Bank Small Grants Program funded an international seminar and workshop to disseminate the results of the project and other work on elasmobranchs throughout the region. Participants attended from 13 countries, with over 30 papers presented. The workshop sessions were used to discuss conservation and management issues in Sabah and elsewhere in the region, and a number of important recommendations were made.

## RESULTS

The workshop proceedings and project report (Fowler, in press) will include several papers presenting more detailed information on the results of the project. Only a brief summary is presented here.

### Biodiversity study

Prior to this project, there had been no dedicated detailed study of sharks and rays in Sabah or elsewhere around Borneo or in Malaysia. Some 47 elasmobranch species records were found in the published or unpublished literature reviewed, including some records from the beginning of the century (Manjaji, in press b). There were certainly no published records of freshwater species from Sabah, and only two species of freshwater ray had been recorded in literature from the whole of Borneo. Indeed, most DoF and WWF staff had been unaware that any freshwater elasmobranchs occurred in Sabah.

Regular market surveys very quickly began to yield new records. For example, a *Mustelus* sp. recorded during the first few days of the project was not only a new species record for Sabah, but also the first record of this genus in the state (this and another *Mustelus* species may prove to be undescribed once the genus has been revised). Some problems were encountered with the identification of species in fish markets, because large specimens were quickly cut up and sharks were usually finned before being put on sale. It was particularly difficult to identify large carcharinids to species level when only a head or a photograph of a large finned shark was available for reference. Large numbers of newborns were often seen; some of these and the discarded fetuses whose fins and teeth were not fully developed were also difficult to identify. Several batoid species obtained in markets are identified in table II by 'resurrected' names, based on concurrent studies by Last, Compagno, and Manjaji (e.g., *Himantura pastinacoides*, *H. uarnacoides*). In addition, several others (i.e., *H. gerrardi*, *H. uarnak*, *Pastinachus sephen*) appear to form species complexes which still need to be resolved.

Almost all records were of inshore, coastal species; only one record of a deepwater shark was made (there are no registered deep-water fishing vessels operating in the study area, although depths reach 2,000 m fairly close to the coast). One oceanic species, *Carcharhinus falciformis*, was only observed during visits to an offshore island (Mabul) where a seasonal long-line fishery targeted sharks. Although abundant in landings there, it was not recorded in mainland market surveys. Some coastal species were only obtained from the catches of the Bajau Laut, sea gypsies, subsistence fisher folk who only visit land to trade. They may use different methods and target different habitats from the commercial vessels that regularly land catches for sale in markets.

All freshwater species records were first obtained from Orang Sungei fishermen on the Kinabatangan River, who saved small specimens of the giant freshwater stingray *Himantura chaophraya* (Fig. 2) and several juveniles of an undescribed species of river shark *Glyphis* sp. (Fig. 3, provisionally named the Kinabatangan River shark) for the survey team. The survey team subsequently obtained one newborn river shark from the location indicated by the villagers. Photographs were provided of a juvenile freshwater sawfish *Pristis microdon* and the specimen's rostrum and fins were saved for the project team. Villagers also donated several dried saws of *Pristis microdon* and the green sawfish *Pristis zijsron* to the collection. It is obvious from fishermen's descriptions that the project did not obtain photographs or specimens of all the freshwater and estuarine rays recently reported in the Kinabatangan River. There is at least one other stingray present

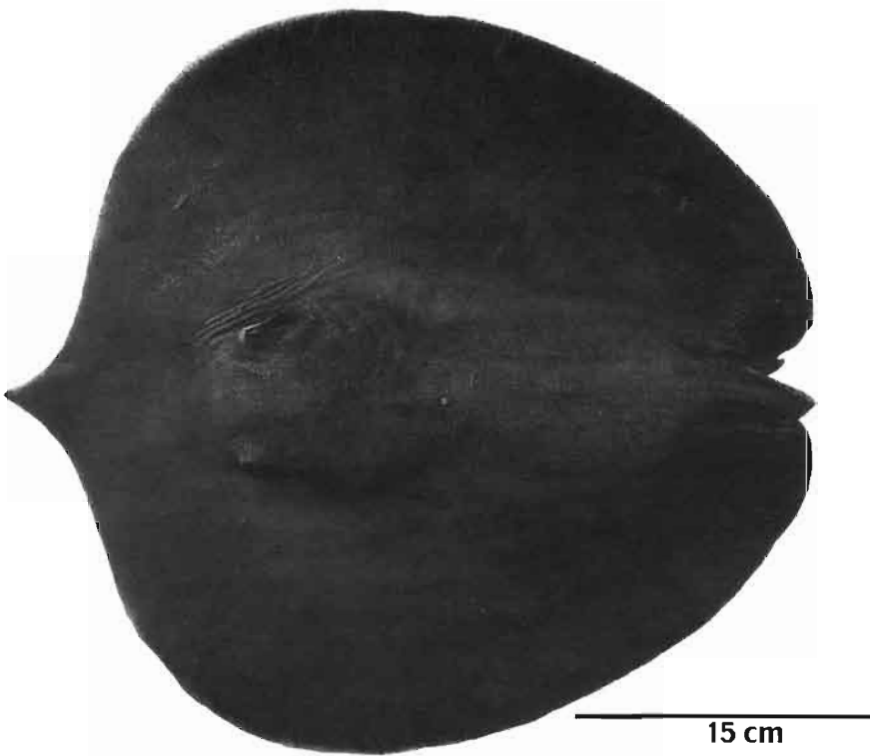


Fig. 2. - Giant freshwater stingray, *Himantura chaophraya* Monkolprasit & Roberts, 1990, from Sabah, East Malaysia. Photo: Mabel Manjaji.



Fig. 3. - Borneo River shark, *Glyphis* sp.B [Compagno, Mycock, Cavanagh, Manjaji & Fowler] from Sabah, East Malaysia. Photo: Scott Mycock and Rachel Cavanagh.

upriver over 200 km from the sea. Other species occur near the river mouth, and very large guitarfish (possibly *Rhinobatos typus* the giant shovelnose ray) are reported to have been caught occasionally over 100 km upstream.

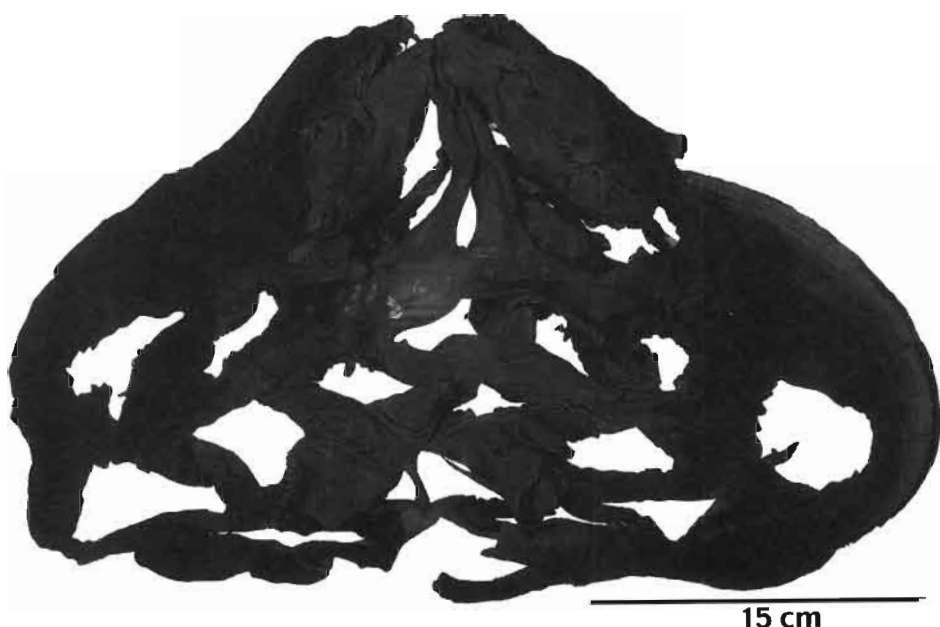


Fig. 4. - Borneo wobbegong, *Orectolobus* sp. [Last, Manjaji & Fowler], from Sabah, East Malaysia. Photo: Thor Carter.

After eighteen months, 69 species had been recorded; 34 sharks and 35 rays. Twenty-three of these species (14 sharks and 19 rays) were new records for Sabah. At least one species collected was undescribed, the river shark *Glyphis* sp. (Fig. 3), and possibly the Borneo wobbegong (Fig. 4, known only from a mutilated, sun-dried specimen). Several other sharks determined only to genus may also prove to be undescribed, and three rays were found to have more than one morphological form, possibly including some new species. A few species are apparently rare or restricted in range to around Borneo. Some of these may be particularly vulnerable to fisheries. Not all of the 47 species previously recorded in the region had been reconfirmed at the close of the study. However, new species records for Sabah were made throughout the study, including during the last month of the project, and it is likely that more of these missing species and other species not previously recorded from the state will be reported in future. It was recognised that elasmobranch biodiversity in the region was particularly high, having exceeded researchers' expectations during the short study.

An extensive reference collection of sharks and rays was collected and curated, and will be maintained by the Sabah State Museum. Duplicate specimens are being sent to some major fish collections abroad.

The full species list produced (Table II) will be combined with a chondrichthyan fish list for the whole South China Sea area and published as a technical report. Species data from the Darwin Project, including photographs, will also be incorporated in ICLARM's FishBase, thus made more widely available.



### **Socio-economic study**

The two villages studied were predominantly made up of subsistence fishers, with fresh fish being a major component of the diet and few other occupations being available to the villagers. A few fishermen target elasmobranchs, particularly at certain seasons, but almost all catch sharks and rays as bycatch. Surplus ray meat and some shark meat is dried, with fresh meat eaten by the fishermen's families. Lower value shark meat is usually discarded in season, but the fins are very valuable. The white-spotted wedgefish or guitarfish *Rhynchobatus* sp(p). and sawfishes are also targeted for their high value fins. These products and other saleable fish catches are usually taken to fish traders in the village, who transport them to Sandakan for resale. Fish traders also act as money lenders and may supply fishermen with boats and other gear, repaid with a portion of their catches. The study was hampered by the lack of written records in the villages on catch weights, and poor recall by fishermen on details of their catches (Almada-Villela, in press).

### **Education and training**

The Sabahan Project Officer (M. Manjaji, one of the authors) received valuable 'on the job' training from visiting taxonomic and fisheries experts. She is now continuing her research (undertaking an MSc thesis at the University of Tasmania on ray taxonomy) and will return to a post as tutor at the University Malaysia Sabah's Borneo Marine Research Unit (BMRU), thereby continuing the education of undergraduates and related research work through the BMRU in future years.

Several local fishermen and villagers spent a lot of time with the project team, becoming particularly interested in and supportive of the project and its conservation aims. This was helped by the media interest in the project, particularly the "discovery" of the river shark, in state, national papers and international magazines. Key village heads and elders were familiarised with the project and distribution of leaflets and posters will help to maintain and extend this interest, particularly through the continued work of WWF and the DoF in the lower Kinabatangan region.

### **International seminar and workshop**

The final workshop produced a detailed set of conclusions and recommendations, to be published with the proceedings. The State Minister for Agriculture and Fisheries expressed support for the work undertaken by the project, and for follow-up work by the Department of Fisheries and the University Malaysia Sabah in collaboration with local research institutions and the international scientific community.

## **DISCUSSION**

The Darwin Elasmobranch biodiversity project in Sabah is an example of how a collaborative project can call on the expertise of an international network of specialists to provide a considerable boost to local, regional and international knowledge of and interest in a taxonomic group.

Prior to commencement of the project, there was only limited appreciation of the particular life history characteristics and hence management requirements of elasmobranchs in fisheries in Sabah and elsewhere in Malaysia. Information on landings and trade was, in common with most countries, recorded only as 'sharks' or 'rays' rather than at any more detailed taxonomic level, and elasmobranchs had only received superficial

attention during marine resource surveys. The value and uses of sharks and rays by local subsistence fishing communities had not been assessed, neither were elasmobranchs being considered when formulating marine or freshwater fisheries or nature conservation management objectives, or in the context of their importance for the significant dive-tourism industry in parts of Sabah.

By the end of the project, there was a high level of awareness of the special nature of shark and ray populations and their conservation and management requirements among fisheries managers, researchers, non-governmental organisations and the local community, and considerable media coverage had been obtained for the project. Detailed information had been extracted from DoF records (Busing, unpubl. data) to describe elasmobranch fisheries in the State. There was heightened enthusiasm for continuing to build on the results of the project, for example by continuing to record unusual species and developing new elasmobranch research proposals. The importance of maintaining research and reference collections and improving taxonomic training was understood.

An important issue highlighted was how poorly known were many species of elasmobranchs present, particularly the rays. There are obviously numerous taxonomic problems still to be resolved before all species present could be identified. Many genera require detailed review, and the preparation of a catalogue of batoids of the world is of high priority. In the absence of this publication, it will be extremely difficult for researchers and fisheries managers to undertake similar studies elsewhere in Southeast Asian and the Indo-Pacific Region without considerable input from overseas taxonomic experts. This is unfortunate, as rays could be an important indicator of mixed species fishery health (because of their susceptibility to overfishing) and of inshore and freshwater habitat quality. There are likely to be several regional endemics present, including inshore species and the deepwater chondrichthyans, which had not been studied during the project. Some of these species are likely already to have been overfished elsewhere in the Indo-Pacific region.

Freshwater elasmobranchs in Southeast Asia and elsewhere are imperfectly known, very restricted in distribution, and particularly vulnerable to habitat degradation and destruction (Compagno and Cook, 1995). The Kinabatangan River is unusual in that a sizeable amount of its catchment is protected by wildlife conservation legislation. This should provide a key foundation for conservation of the river's freshwater elasmobranchs (Payne and Andau, in press), while the existence of "flagship" species such as the river shark *Glyphis* sp. and sawfish *Pristis microdon* should help to support future conservation initiatives for the whole catchment.

The project has highlighted the value of working closely with local fishermen and other villagers to obtain specimens of rare or unusual species, while at the same time involving the community in conservation and management issues and increasing their awareness of the international significance of freshwater elasmobranchs and their environment. Care was taken not to create an artificial market for rarities during the study. Fishermen were asked not to target them for collection, but only to keep those specimens for the project which were found as dead bycatch, which would otherwise have been discarded or used for food.

Finally, the funding for a final international seminar and workshop and for publication of the proceedings was essential to enable the results of the project to be widely disseminated and allowing participants to reach a consensus over priorities for future research, conservation and management efforts.

**Acknowledgements.** - Funding for this project was mainly provided by a grant from the Darwin Project for the Survival of Species to the IUCN Species Survival Commission Shark Specialist Group. The Darwin Initiative is a unique UK venture for funding collaborative projects that will help to conserve global biodiversity. It funds collaborative surveys, research projects, training programmes and other work in centres of biodiversity, and is part of the UK Government's commitment to the aims of the Biodiversity Convention.

However, the study could not have been undertaken without the enthusiastic and invaluable support of the Department of Fisheries - Sabah. We particularly acknowledge the contribution of the Director, Mr Yap Kon Shen, Mr Alvin Wong, and many staff at the Fisheries Research Centre and regional offices. The study benefited greatly from the advice of Dr Junaidi Payne of WWF-Malaysia and the assistance of WWF staff with fieldwork. Dr Ridzwan Abdul Rahman, Head of the Borneo Marine Research Unit of the University Malaysia Sabah (UMS), allowed the second author to continue work on the Darwin Project after joining his staff. The Nature Conservation Bureau provided essential administrative support.

Valuable contributions were provided by volunteer John Denham and several other members of the Shark Specialist Group: Patricia Almada-Villela, Sid Cook, Tyson Roberts, and Gordon Yearsley. The final meeting was partly funded by a contribution to UMS from the World Bank Small Grants Program, and was organised and run by the DoF and Institute of Development Studies (Sabah). Finally, we are most grateful for the generous help and hospitality provided by many Sabahan villagers, market stall holders and fishermen.

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## FUNDAMENTAL ZONATION IN ELASMOBRANCH OVIDUCAL GLANDS

by

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**ABSTRACT.** - Oviducal glands (OG) in elasmobranchs, variously termed shell or nidamental glands, are specialized regions of the anterior oviduct modified to perform the following functions: 1) lubrication to facilitate transmission of the egg, 2) generation of ciliary beating to assist in moving the egg; 3) production of a jelly coat around the egg, 4) production of the various egg investments, in species that have coverings, and 5) serve as a sperm receptacle. Previous terminology applied to OG have inferred function, such as albumen, mucous and shell zone. In the case of the jelly coats that envelope the egg, the term albumen is not appropriate since the precise composition of the jelly coats is unknown. Similar uncertainty exists regarding the elaborations of the "mucous" zone. In our study of OG from elasmobranchs with various modes of reproduction, we have identified a constant pattern of morphology that exists across reproductive modes. In order to facilitate discussion of OG across reproductive types and to be able to distinguish similar zones among different species, we have established terminology based on the appearance of the epithelium when viewed in longitudinal section. The OG has similar zonation, i.e., 1) club zone, 2) papillary zone, 3) baffle zone, and 4) terminal zone. All species thus far studied have the same fundamental organization with the exception of the yellow spotted stingray, *Urolophus jamaicensis*, which is aplacental with trophonemata and is unusual in that it does not form any type of egg covering except for mucous and jelly and lacks a baffle zone. In oviparous species such as *Raja eglanteria*, a hard, permanent egg case is produced that is deposited externally. In aplacental yolk sac species such as *Squalus acanthias*, a transient, flexible "candle" case is produced and retained *in utero* until it disintegrates. In placental species such as *Mustelus canis*, a pliable egg envelope is formed that is maintained throughout gestation and is incorporated into the placenta. Species that produce an egg case, candle case and egg envelope all share similar design features of the baffle zone or egg investment zone, e.g., a blown extrusion die complex that manipulates liquid crystal secretions of gland tubules in a spinneret region of the baffle zone to form complex lamellated structures. Secretory units eject their products into transverse grooves that extend across the width of the gland. Adjacent secretory units thus form a part of each subsequent layer of the covering. Species differences in OG architecture are noted.

**RÉSUMÉ.** - Zonation fondamentale des glandes nidamentales chez les élamobranches.

Les glandes de l'oviducte (OG) des élamobranches, aussi nommées glandes nidamentales, sont des organes spécialisés ayant plusieurs fonctions: 1) la lubrification pour faciliter la transmission de l'oeuf, 2) la production du battement ciliaire qui aide au mouvement de l'oeuf, 3) la production d'un revêtement gélatineux autour de l'oeuf, 4) la production des enveloppes de l'oeuf (chez quelques espèces), et 5) servir de réceptacle pour le sperme. Jusqu'à présent, la terminologie utilisée pour les OG se référait aux fonctions, comme par exemple, l'albumen, les zones à mucus et la capsule ovigère. Dans le cas du revêtement gélatineux, le terme d'albumen n'est pas adapté puisque la composition de

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cette gélatine reste inconnue. Le terme de zone à "mucus" relève de la même incertitude. Lors de nos études sur des élamobranthes dont les modes de reproduction sont variés, nous avons remarqué une constance dans l'organisation structurale. Pour pouvoir comparer les différentes espèces, nous avons établi une terminologie fondée sur l'apparence de l'épithélium en coupe longitudinale. Les OG ont une zonation similaire: 1) une "zone club", 2) une zone papillaire, 3) une "zone baffle" (zone de recouvrement), et 4) une zone terminale. Toutes les espèces étudiées jusqu'à présent ont une organisation fondamentalement similaire sauf *Urolophus jamaicensis* qui ne produit autour de l'oeuf qu'un revêtement gélatineux et des couches de lubrification. Chez les espèces ovipares comme *Raja eglanteria*, une enveloppe solide et permanente est produite et déposée à l'extérieur. Chez les espèces aplacentaires à sac vitellin comme *Squalus acanthias*, une membrane souple, en forme de bougie, est formée et maintenue *in utero* jusqu'à sa désintégration. Chez les espèces placentaires comme *Mustelus canis*, une enveloppe souple est formée, maintenue pendant toute la gestation et incorporée dans le placenta. *Urolophus jamaicensis*, qui est aplacentaire avec formation de trophonemata (villosités utérines), est inhabituel car il ne forme aucune sorte de recouvrement de l'oeuf mis à part le mucus et la gélatine, et ne possède pas de "zone baffle". Les espèces qui produisent des enveloppes, partagent des structures semblables au niveau de la "zone baffle"; celle-ci comporte une matrice complexe d'extrusions turgescentes qui forment des structures lamellaires complexes avec les sécrétions cristallines liquides produites par des tubules glandulaires situés dans une région réticulée de la "zone baffle". Les unités sécrétrices émettent leurs produits dans des rainures transversales qui s'étendent sur toute la largeur de la glande. Les unités adjacentes forment les couches suivantes de l'enveloppe. Les différences spécifiques de l'architecture des OG sont données.

Key-words. - Elasmobranchii, Oviducal glands, Shell, Egg case, Candle, Egg envelope, Oviduct.

Sharks, skates, and stingrays constitute the elasmobranch fish. These cartilaginous fishes display a varied array of reproductive strategies ranging from oviparity to several modes of viviparity (Hamlett, 1987, 1989; Hamlett *et al.*, 1993). All employ internal fertilization and have a typical repertoire of vertebrate hormones that mediate sexual cycles and gonadal maturation. Oviparous species such as the clearnose skate, *Raja eglanteria*, release egg cases containing fertilized eggs into the sea where they will develop at the expense of yolk sequestered in their yolk sac. Viviparous species have a variety of methods for nourishing their young. Some sharks such as the aplacental spiny dogfish, *Squalus acanthias*, are completely dependent for nutrition upon the yolk sequestered by the mother in the egg. In the smooth dogfish, *Mustelus canis*, initial development relies on yolk sac contents. After this yolk dependent phase, the yolk sac becomes modified into a yolk sac placenta that provides for nutrient and respiratory exchange during the remainder of gestation (Hamlett *et al.*, 1985a, 1985b, 1985c; Hamlett, 1987, 1989; Hamlett *et al.*, 1993a, 1993b, 1993c, 1993d). Other modes of viviparity include uterine villi known as trophonemata in stingrays that secrete histotroph, also known as "uterine milk" which the developing embryos ingest (Hamlett *et al.*, 1985e; Hamlett *et al.*, 1996a, 1996b) and the practice of oophagy by several large sharks such as the porbeagle shark, *Lamna nasus*. Throughout pregnancy, the mother continues to ovulate eggs which the young ingest. In addition to oophagy, the sandtiger, *Carcharias taurus*, practices intra-uterine embryonic cannibalism in which more developmentally advanced embryos feed on immature siblings (Gilmore, 1993; Hamlett *et al.*, 1993b).

Historically the terms shell, nidamental and oviducal gland have been used imprecisely and interchangeably. The region of the oviduct that produces a tough egg case that is deposited to the exterior in oviparous species is correctly termed the shell gland since this term denotes its function. A shell is defined as a hard, outer covering, hence the des-

ignation. The term nidamental gland is best used to refer to the thin egg coverings of viviparous species. The term nidamental is derived from *nidus* (L.) for nest. In many placental species each embryo is surrounded by its own egg covering, and the embryo and its coverings develop in their own uterine compartment, hence the nest. Neither of the previous terms can be correctly applied to the gland when referring to the region of the oviduct in some rays where no egg covering is produced. To establish a consistent terminology, we have chosen not to use the terms shell or nidamental but to use only the term oviducal gland (OG) to refer to any of the aforementioned glands since they are all derived from the oviduct.

Early descriptions of OG in elasmobranchs were given by Borcea (1906), Widakowich (1906), Filhol and Garrault (1938), Nalini (1940), and Prasad (1945, 1948). The vast majority of the work on elucidating the structure and function of elasmobranch OG has centered on the oviparous dogfish, *Scyliorhinus canicula*. Metten (1939) described the structure of the gland and several authors considered its histochemical characteristics (Threadgold, 1957; Rusaouën 1976a, 1978). Krishnan (1959) added information on the histochemistry of the OG in *Chiloscyllium griseus*. The formation and nature of the components of the egg capsule of *S. canicula* has been well studied (Knight and Hunt, 1976; Rusaouën 1976b, 1986; Rusaouën-Innocent, 1985, 1989, 1990; Hunt, 1985; Feng and Knight, 1992, 1994a; Knight *et al.*, 1993; Knight and Feng, 1992, 1994b; Hepworth *et al.*, 1994; Knight, 1996). Thomason *et al.* (1994) have investigated the antifouling properties of the egg case and Koob and Cox (1993) have reported on tanning of the egg capsule in the little skate, *Raja erinacea*. Pratt (1993) has discussed sperm storage in the OG of some elasmobranchs.

## MATERIAL AND METHODS

Female clearnose skates (*Raja eglanteria*), spiny dogfish (*Squalus acanthias*), and smooth dogfish (*Mustelus canis*) were obtained via long line from shallow waters in the Chesapeake Bay. Female yellow spotted stingrays (*Urolophus jamaicensis*) were obtained via handnets in shallow waters off Long Key, Florida. Females were anesthetized with MS 222, humanely sacrificed by cervical transection and pithing, and opened by a longitudinal ventral incision and the oviducal glands (OGs) isolated. Glands were fixed in 10% neutral buffered formalin. Samples were dehydrated through a graded series of alcohols and embedded in JB-4 glycol methacrylate (Polysciences). Two micrometer thick sections were cut with glass knives and affixed to glass slides. Sections were stained with either toluidine blue, periodic acid-Schiff, or methylene blue-basic fuchsin. Sections were photographed on a Nikon Optiphot-2 light microscope equipped with a Microflex AFX-DX photomicrographic attachment.

## RESULTS

The elasmobranch oviducal gland (OG) is a specialized swelling in the anterior oviduct ranging in size from the small *Squalus* gland which is 1 cm wide and 1.5 cm long to the larger *Raja* gland which is 4 cm wide and 2.5 cm long. In oviparous species, including *Scyliorhinus canicula*, *Raja eglanteria* and *R. erinacea* the gland is roughly heart-

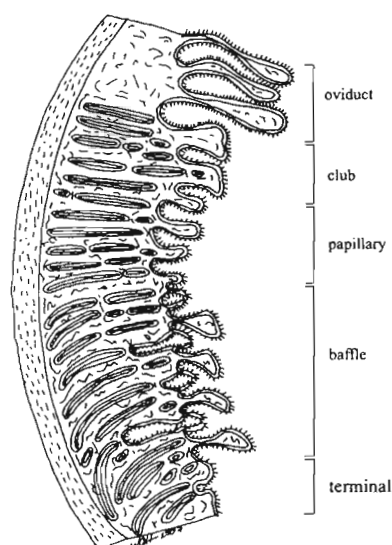


Fig. 1. - Composite line drawing of a "generic" elasmobranch OG. The oviduct leads to the gland which have fundamental zones common to all species thus far studied: the club, papillary, baffle and terminal zones.

shaped with the upper wings extending toward the ovary and the base of the heart directed toward the posterior oviduct. In *Squalus acanthias*, a viviparous aplacental yolk sac type, and *Urolophus jamaicensis*, a viviparous aplacental with trophonemata, the gland is small and barrel-shaped. In viviparous placental species, including *Mustelus canis*, the superior wings of the gland are extended and wind like a ram's horn.

Four histologically recognizable zones are common to all elasmobranch OG (Fig. 1). Even in species that do not produce an egg investment except for the jelly coat and mucous, i.e., *U. jamaicensis*, the same zone equivalents are maintained, albeit in a modified form. The specializations of the gland in *U. jamaicensis* will be dealt with in a separate study. Previous descriptions of the zonation in *S. canicula* have reported five to nine zones depending on the author and the means of analysis, i.e., histochemistry, morphology via scanning electron microscopy, etc. (Threadgold, 1957; Rusaouën 1976a, 1978). They have generally been designated A, B, C, etc. From our survey we recognize four fundamental zones present in all species. Additional sub-zones occur in some particular species but the fundamental zonation is maintained. We have chosen to designate the basic zones on the basis of the histological appearance of the surface layer. This is purely descriptive and is not intended to suggest function. By adopting a uniform terminology that can be applied to all OG, regardless of the type of reproduction, we can easily distinguish equivalent zones between species. This classification may be modified later as

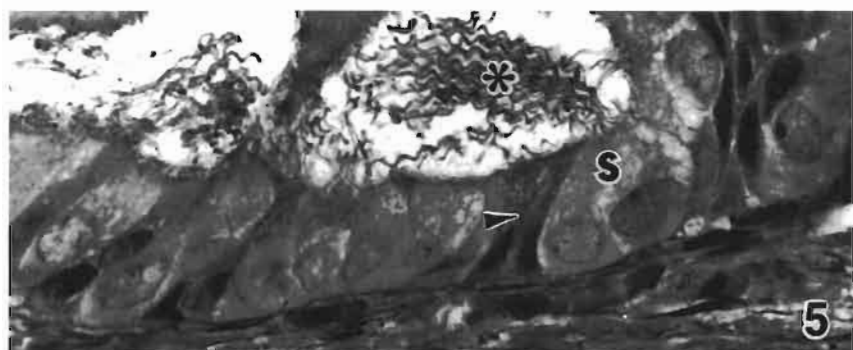
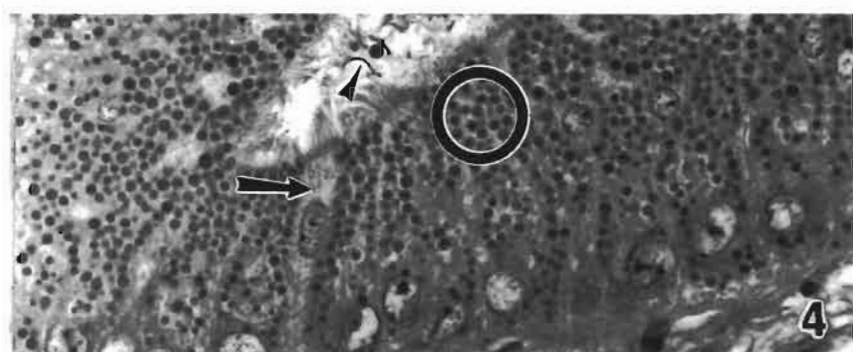
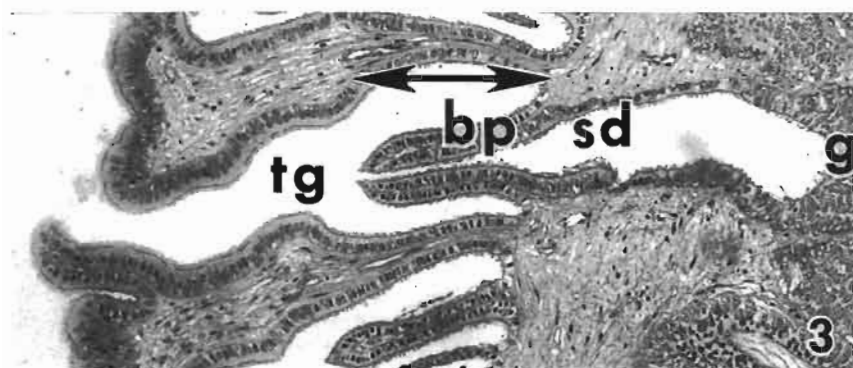
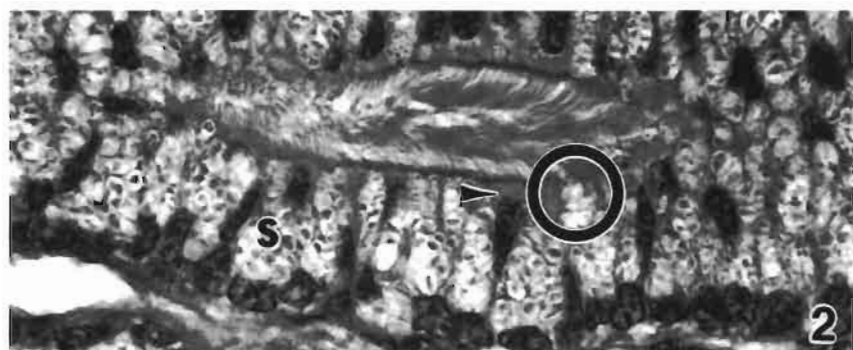
Fig. 2. - Club zone of *R. eglanteria*. Pyriform sustentacular cells with cilia (arrowhead) are interspersed with granulated secretory cells (s) in the process of secretion (circle). 600x.

Fig. 3. - Baffle zone of *R. eglanteria*. Gland tubules (g) lead to the secretory duct (sd) which is confluent with the spinneret region (double headed arrow) with baffle plates (bp). Secretory material is then delivered to transverse grooves (tg). 600x.

Fig. 4. - Gland tubule from baffle zone of *R. eglanteria*. Pyriform ciliated sustentacular cells (arrow) alternate with columnar secretory cells with apical secretory vesicles (circle). Individual sperm (arrowhead) are occasionally present in the gland lumen. 600x.

Fig. 5. - Terminal zone of *M. canis*. Thin columnar sustentacular cells alternate with engorged secretory cells (s). Sperm bundles (asterisk) occur in the gland lumen. 600x.





particular functions are more precisely elucidated for each zone. We will give a description here of a "generic" OG and comment on the variability of the zones in different species.

In all species thus far studied, the anterior oviduct is composed of longitudinal folds which extend to the top of the gland. The ciliated columnar epithelium helps move the egg into the OG proper. This is effected by the action of the two cell types present, i.e., mucous and ciliated. Mucous cells have the general appearance of typical goblet cells. Secretory material engorges the cell apex while the nucleus is basal. Ciliated cells are interspersed with mucous cells but mucous cells predominate in the crypt between folds where they are PAS-. Ciliated cells are pyriform and their nuclei are located toward to lumen. PAS+ secretory granules occur in many of the columnar cells of the surface epithelium.

The club and papillary zones comprise what has previously been called the albumen zone (Prasad, 1945). The club zone is characterized by squarish surface folds of the epithelium which in cross section are club shaped. Surface epithelium of the club is ciliated, simple columnar. Gland tubules are confluent with the grooves between adjacent folds. The tubules are characterized by two types of cells, i.e., secretory and sustentacular (supportive). Secretory cells are columnar with basal nuclei. They contain secretory vesicles which have a dense core granule surrounded by a light halo (Fig. 2). The granule is generally PAS+ while the halo is PAS-. This makes the entire zone appear to be very PAS+. Small triangular shaped ciliated sustentacular cells also occur. Their nuclei are pyriform and located toward the lumen. The number of club zone rows in *R. eglanteria*, *S. acanthias* and *M. canis* is given in table I.

Oviducal glands are devoid of smooth muscle. The papillary zone, in *R. eglanteria*, is characterized by low relief conical projections. The surface epithelium is simple columnar with cilia. Simple tubular glands empty their secretions in the groove between adjacent papillae. Secretory cells are simple columnar with homogeneous secretory vesicles. The vesicles are PAS+ but are less PAS+ than those of the club zone. While it might appear that the papillary zone is simply a gradual change from the club zone this is not the case. In *Raja* there is a definite change in the staining characteristics of the tubules of the respective zones. Sub-zones of the papillary region occur in both *Squalus* and *Mustelus*, based on staining affinity with various dyes. These differences will be elucidated in forthcoming publications dealing with individual species. Alternating with the secretory cells are pyriform ciliated cells. The number of rows in the papillary zone of *R. eglanteria*, *S. acanthias* and *M. canis* is given in table I.

The baffle zone is distinctive (Fig. 3). Apically flattened surface plateaus alternate with transverse grooves that extend across the gland. Simple tubular glands, composed of secretory and ciliated sustentacular cells, are confluent with secretory ducts composed exclusively of ciliated columnar cells. Secretory granules in gland tubules are entirely PAS-. There is no halo associated with the regularly shaped dense core granules (Fig. 4). Secretory material from the glands are passed to the spinneret region of the baffle zone. In *S. canicula*, each spinneret is composed of two baffle plates (Knight and Feng, 1992).

Table I. - Number of rows per zone in oviducal glands (OGs).

	Club	Papillary	Baffle	Terminal
<i>Raja eglanteria</i>	12	21	27	Sparse and shallow
<i>Squalus acanthias</i>	13	14	27	Extensive
<i>Mustelus canis</i>	2	16	12	Few and deep

which project into the base of each transverse groove on either side of the opening of the secretory duct. The same arrangement occurs in both *R. erinacea* and *R. eglanteria* (Fig. 3). Baffle plates are flattened when viewed from the side and are composed of densely ciliated columnar epithelium. Baffle plates orient the secretory products going into the transverse grooves, such that the layers of the egg covering are laid down at angles (Knight, 1996b).

There are species differences in the anterior tubule layers in this zone. For example, in *Squalus* the first four to five layers of tubules stain heavily with both toluidine blue and methylene blue-basic fuchsin. These tubules represent a thin whitish band that is visible on the gland's outside surface when viewed grossly. In *Mustelus*, the first layer of tubules has different staining affinity for methylene blue-basic fuchsin than the next two to three tubules, which in turn are different from the bulk of the zone. The particular details of OG organization in both *Squalus* and *Mustelus* will be presented elsewhere.

Occasionally individual sperm are seen in the tubules of the baffle zone in *Raja* (Fig. 4) and *Mustelus*. These sperm, however, are isolated and are not associated in bundles. These appear to be incidentally present and do not represent sperm storage *per se* in this zone.

The terminal zone shows some of the greatest variation amongst the elasmobranchs but there are many shared features. In contrast to earlier zones with abundant tubules, the terminal zone is primarily connective tissue with few shallow, simple tubular glands extending into the connective tissue. Blood sinuses are present beneath the columnar cells which make up the epithelium. Secretory tubules are more common immediately after the baffle zone but quickly become increasingly rare. These tubules are composed of two cell types. First, and most common, are PAS- secretory cells. There are also darker staining cells that resemble those of the baffle zone. In *Mustelus*, terminal zone tubules extend more deeply into the connective tissue than in the other species studied and ordered aggregations of sperm are observed in the gland tubules far distant from the lumen (Fig. 5). In *Raja* the terminal zone has what appear to be mucous and seromucous secretory cells. In *Squalus* the terminal zone extends all the way to the uterus. This segment has been described as the posterior oviduct but we feel this is more properly thought of as a prolongation of the OG. Columnar secretory cells have frothy secretions that are mildly PAS+.

## DISCUSSION

Adaptations of the female reproductive tract allow varied modes of reproduction in elasmobranchs. The oviducal gland of elasmobranch fish is a complex structure formed as a hypertrophied region of the anterior oviduct. It generally consists of two identical dorsal-ventral halves with flattened luminal surfaces. The gland is generally constructed from a large number of unbranched tubular glands which lead to secretory ducts. The club and papillary zones add material to coat the egg such as mucous and various components of the egg jelly. Secretory material in the baffle zone is then passed to the spinneret region where paired baffle plates manipulate the secretion to produce the various layers or lamellae of the egg investment. A series of transverse grooves extend across the gland lumen and receive secretory material from the spinneret region. Each transverse groove in the baffle zone is responsible for the elaboration of each individual layer of the egg covering (Borcea, 1906).

The OG of elasmobranchs which produce egg coverings is highly conserved. Variation in what is being secreted by the tubules and the relative length of the various zones seems to be the primary way that different elasmobranchs produce different egg coverings. In *Raja*, the baffle zone dominates the gland while the club zone tubules are much longer than the papillary zone tubules. In contrast *Squalus* and *Mustelus* have shorter baffle zones while the papillary zone is approximately twice as large as the club zone. Differences such as these may allow *Raja erinacea* to produce a hard egg case with six types of protein in the egg case wall (Koob and Cox, 1993) while *Squalus* produces a thin, transient, flexible case which forms a "candle" around the developing embryos. *Mustelus* produces a thin plaited egg envelope which will be drawn around the embryo as it develops and will accommodate the placenta. The blue shark, *Prionace glauca*, is also placental and its gland (Pratt, 1979) closely resembles that of *Mustelus*. In *Mustelus*, the club zone is much shorter than the papillary zone, the baffle zone has relatively short tubules compared to *Raja*, and the terminal zone is similar both in the organization of the connective tissue and the presence of blood sinuses beneath the epithelium. Baffle plates, previously termed zottehan (Widakowich, 1906), oblique plates (Metten, 1939), and tufts (Prasad, 1945), are absent in the yellow spotted stingray *Urolophus jamaicensis*. This stingray does not produce an egg covering except for the jelly coat and mucous.

Efforts to further characterize the various functions of OG in elasmobranchs is currently underway in our laboratory. We are extending our investigations to include OG from oophagous animals and stingrays that do form egg coverings. A comprehensive comparative synthesis of OG structure and function in elasmobranchs of varying reproductive modes is the goal. We are interested in the fate of sperm in the female reproductive tract, especially in light of the simultaneous functions occurring including fertilization, production of jelly coats to invest the egg and production of various types of egg coverings, as well as sperm motility, viability and storage.

**Acknowledgements.** - We wish to thank Dr. John A. Musick, Virginia Institute of Marine Sciences, for providing access to specimens. This study was supported by funds provided by Indiana University School of Medicine.

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## SPERM AGGREGATION AND SPERMATOZEUGMATA FORMATION IN THE MALE GENITAL DUCTS IN THE CLEARNOSE SKATE, *RAJA EGLANTERIA*

by

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**ABSTRACT.** - The process of spermatogenesis in elasmobranchs yields a spermatocyst composed of a Sertoli cell and its cohort of associated spermatozoa laterally bundled and embedded in the apical end of the Sertoli cell. When the liberated Sertoli cell and the spermatozoa reach the first major segment of the extratesticular duct system, the proximal epididymis, the sperm have disaggregated and are present as individual sperm surrounded by a heterogeneous suspension consisting of: 1) membrane limited vesicles derived from Sertoli cells (Sertoli cell bodies), 2) Sertoli cell cytoplasts which are remnants of Sertoli cells exclusive of Sertoli cell bodies, 3) large Leydig gland bodies, and 4) secretions and/or cell fragments of epithelial cells of the genital ducts. The principal extratesticular genital ducts of male *Raja eglanteria* consist of the paired: 1) epididymes, 2) ductus deferens, and 3) seminal vesicles. In concert, they function to: 1) transport spermatozoa, 2) contribute to the seminal fluid, and 3) effect sperm aggregation. The epididymes and ductus deferens receive secretions from the Leydig gland (Leydig gland bodies) which are considered the primary source of seminal fluid. The epididymis is composed of ciliated columnar cells. Cranially, luminal contents of the epididymis include sperm laterally aligned head-to-head in groups of 2-12. In the middle epididymis, large aggregations of laterally aligned sperm are embedded head-first in a homogeneous extracellular mass. Epithelial folds project into the duct lumen and decrease in size in the ductus deferens. Epithelium of the ductus deferens is simple columnar. Leydig secretions here consist of small secretory vesicles. The lumen of the seminal vesicle is massive when compared with the epididymis and ductus deferens. Epithelial shelves of simple columnar ciliated cells project into the lumen and smooth muscle is a component of the vesicle walls. Secretory activity of seminal vesicle epithelium may contribute to the surrounding matrix. Sperm are present as definitive simple spermatozeugmata.

**RÉSUMÉ.** - Agrégation du sperme et formation de spermatozeugmes dans les conduits génitaux mâles de la raie à nez blanc, *Raja eglanteria*.

La spermatogenèse des élamobranches aboutit à un spermatocyste composé d'une cellule de Sertoli et d'une cohorte de spermatozoïdes accumulés latéralement et regroupés au niveau apical de la cellule de Sertoli. Lorsque cet ensemble atteint le premier segment du système tubulaire extratesticulaire, l'épididyme proximal, le sperme s'est désagrégé. Les spermatozoïdes sont indépendants et baignent dans une suspension constituée: 1) de vésicules bordées d'une membrane provenant des cellules de Sertoli (organites des cellules de Sertoli), 2) du contenu cytoplasmique des cellules de Sertoli (autre que les organites), 3) de grandes glandes de Leydig, et 4) de sécrétions et/ou de fragments des cellules épithéliales des conduits génitaux. Le système tubulaire extratesticulaire du mâle de *Raja eglanteria* se compose de structures paires: 1) les épididymes, 2) les canaux déférents, 3) les vésicules séminales.

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Leurs rôles sont: 1) le transport des spermatozoïdes, 2) la contribution à former le liquide séminal, et 3) l'accomplissement de l'aggrégation du sperme. Les épидидymes et le canal déférent reçoivent les sécrétions des glandes de Leydig. Ces sécrétions sont considérées comme la composante majoritaire liquide séminal. Les cellules épithéliales de l'épididyme sont ciliées. Au niveau proximal, le contenu de la lumière de l'épididyme consiste en de larges agrégations de spermatozoïdes, agglutinés latéralement par la tête par groupes de 2 à 12 dans une masse extracellulaire homogène. Des replis épithéliaux se projettent dans la lumière du conduit. La taille de ces replis décroît dans le canal déférent. L'épithélium du canal déférent est simple et columnaire. Les sécrétions de Leydig, à ce niveau, proviennent de petites vésicules sécrétrices. La lumière de la vésicule séminale est massive par rapport à celle de l'épididyme et du canal déférent. Les parois de la vésicule séminale consistent en un épithélium qui présente des invaginations de cellules ciliées simples dans la lumière et de muscle lisse. Il est possible que les sécrétions de l'épithélium de la vésicule séminale contribuent à la matrice entourant les spermatozoïdes, lesquels sont présents en tant que simples spermatozeugmes.

Key-words. - Rajidae, *Raja eglanteria*, Sperm aggregation, Spermatozeugmata, Epididymis, Ductus deferens, Seminal vesicle.

Sperm storage in male elasmobranchs has previously been described (Matthews, 1950; Botte *et al.*, 1963; Stanley, 1983; Mann, 1984; Pratt and Tanaka, 1994) but no discussion of the process of sperm aggregation in the male genital ducts has been presented. This brief note describes the process of sperm aggregation utilizing light microscopy. A detailed presentation of the process using scanning and transmission electron microscopy will appear in a subsequent publication.

Variation exists regarding terminology applied to the genital ducts of elasmobranchs (Borcea, 1906; Matthews, 1950; Botte *et al.*, 1963; Stanley, 1963). From the testis, spermatozoa and Sertoli cell fragments move through the efferent ductules located in the anterior end of the mesorchium. The efferent ductules are single in skates and number four in *Squalus acanthias* (Callard, 1988).

Most authors refer to the highly coiled and convoluted, small diameter initial segment of the extratesticular ducts as the epididymis. This segment is continuous with the broader diameter, sinuous but straighter ductus deferens, also called the vas deferens or Wolffian duct. The ductus deferens is continuous with the folded and large diameter seminal vesicle also referred to as the ampulla of the ductus deferens. Jones and Jones (1982), Jones *et al.* (1984), and Jones and Lin (1993) refer to the derivative of the mesonephric duct as the ductus epididymis rather than the ductus deferens, the term used by most authors.

We have chosen to use the most widely held terminology, e.g., epididymis, ductus deferens and seminal vesicle. Both the epididymis and ductus deferens receive a viscous fluid produced by the adjacent Leydig gland. The two broad seminal vesicles receive no ducts from the Leydig. Each seminal vesicle has a lateral dilation termed the sperm sac. This is a misnomer because these structures do not store sperm but are Marshall's alkaline gland. The seminal vesicles unite to form the single urogenital sinus that ends as the urogenital papilla emptying into the cloaca. Luminal contents of the ductal system include Sertoli cell bodies, Sertoli cell cytoplasm and Leydig gland bodies.

Male elasmobranchs commonly form sperm aggregates as sperm and genital duct secretions are moved down the genital tract. The clearnose skate *Raja eglanteria* conforms to this model. Leydig gland secretions contribute to the matrix material in the epididymis and ductus deferens. Other components of the matrix are cell fragments from testicular



Sertoli cells and genital duct cells. The changing components of the matrix may play a role in sperm aggregation as they are moved down the tract.

## MATERIALS AND METHODS

Male clearnose skates, *Raja eglanteria*, were obtained via long line from shallow waters in the Chesapeake Bay. Males were anesthetized with MS 222, humanely sacrificed by cervical transection and pithing, and opened by a longitudinal ventral incision and the testes and genital ducts isolated. They were placed in 10% neutral buffered formalin and dehydrated through a graded series of alcohols and embedded in JB-4 glycol methacrylate (Polysciences). Two micrometer thick sections were cut with glass knives and affixed to glass slides. Sections were stained with either toluidine blue, periodic acid-Schiff, or methylene blue-basic fuchsin. Sections were photographed on a Nikon Optiphot-2 light microscope equipped with a Microflex AFX-DX photomicrographic attachment.

## RESULTS

The male genital system in *Raja eglanteria* consists of a paired set of organs which lie dorsally in the body cavity. Proximally the genital ducts lie on the Leydig gland and distally are supported by the kidneys (Fig. 1). Sperm and other materials from the testes reach the genital ducts via an efferent duct which leads to the proximal epididymis. The epididymis is a highly convoluted tubule with a small lumen. The ducts gradually become less convoluted and increase in luminal area as they move posteriorly. The ductus deferens is larger in diameter than the epididymis and follows a sinuous rather than convoluted course. The seminal vesicle is the terminal portion of the duct system. It has up to four folds and the terminal portion rests on the alkaline gland.

Leydig glands are a series of branched tubular glands that secrete into the epididymis and ductus deferens (Fig. 2). The Leydig gland is the modified anterior section of the mesonephros that produces and transmits a milky secretion via apical secretory vesicles of the epithelium that congeals into retractile bodies following fixation in formalin (Fig. 2). In immature males urine is formed by these tubules. In *R. eglanteria*, the epithelium is simple columnar. Some of the cells are ciliated while others are secretory.

In *R. eglanteria*, the proximal ductus epididymis is narrow, convoluted and unpigmented when viewed grossly. It has a pseudostratified columnar epithelium with both cilia and microvilli and individual spermatozoa are randomly scattered in the lumen (Fig. 3). No sperm aggregation occurs. Sertoli cell bodies, Sertoli cytoplasm, Leydig gland bodies, and epididymal secretions are present in the matrix surrounding the sperm.

In the distal epididymis, the appearance of the tubules changes slightly as they become larger in diameter. The tubules are separated by less connective tissue and are lined by simple columnar epithelium (Fig. 4). Some of the cells are ciliated with microvilli and others are characterized by apical secretory vesicles. In the distal epididymis sperm aggregates form as groups of two to twelve sperm with their heads laterally aligned (Figs 4, 5). Luminal matrix contents continue to include epididymal cell components, Sertoli cell bodies, Sertoli cell cytoplasm and Leydig gland bodies (Fig. 5). The role these components may play in sperm aggregation or maintenance is unknown.

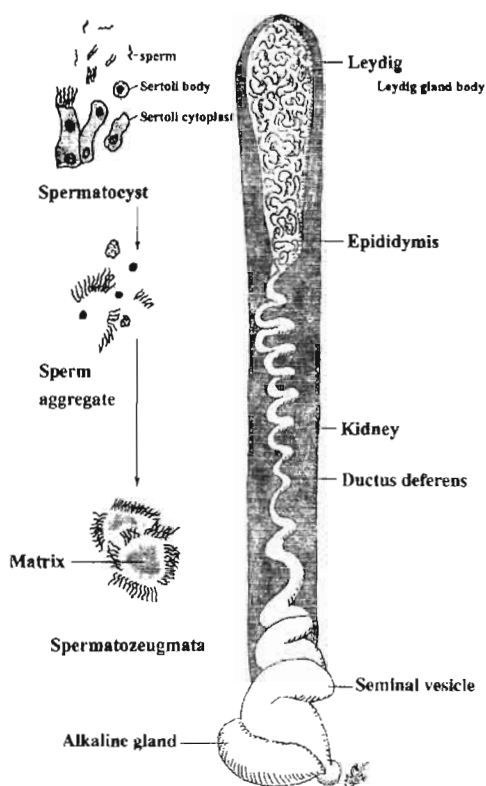


Fig. 1. - Right side: diagram of the major extratesticular ducts of the male *Raja eglanteria* and their relations to the Leydig gland, kidney and alkaline gland. Left side: diagram of sperm disaggregation from the spermatocyst and reaggregation in the extratesticular genital ducts.

In *R. eglanteria*, the epithelium of the ductus deferens is uniformly simple ciliated columnar with microvilli. Sperm associate laterally. They are joined at their heads to form larger aggregates. Matrix material provides the nidus for further sperm aggregation. The source of the matrix material includes residual Sertoli cell bodies, Sertoli cell cytoplasm, Leydig gland bodies, secretory material from the epididymis and/or ductus deferens and as yet unidentified components. In the proximal ductus deferens, large folds and ridges of the epithelium and subjacent mucosa extend into the lumen. The tubule is no longer highly convoluted and is much wider than in the epididymis. Resorption of fluid starts to occur as there is less matrix material in the lumen.

The seminal vesicle is characterized by low, simple columnar to cuboidal epithelium with cilia and microvilli (Fig. 6). Grossly discernable thin walled partitions occur in

Fig. 2. - *Raja eglanteria*. Cross section of a Leydig gland depicting columnar cells (arrow) with apical secretion vesicles (circle) and aggregations of secretory product in the lumen. Secretory vesicle products form circular masses in the lumen (asterisk) which in turn form larger luminal secretory masses (s). 600X.

Fig. 3. - Pseudostratified epithelium of the proximal portion of the epididymis have both cilia and microvilli. Sperm are present as individuals (arrow) and are not aggregated. The matrix suspension (m) includes epididymal secretions, Sertoli cell bodies and Leydig gland bodies. 600X.

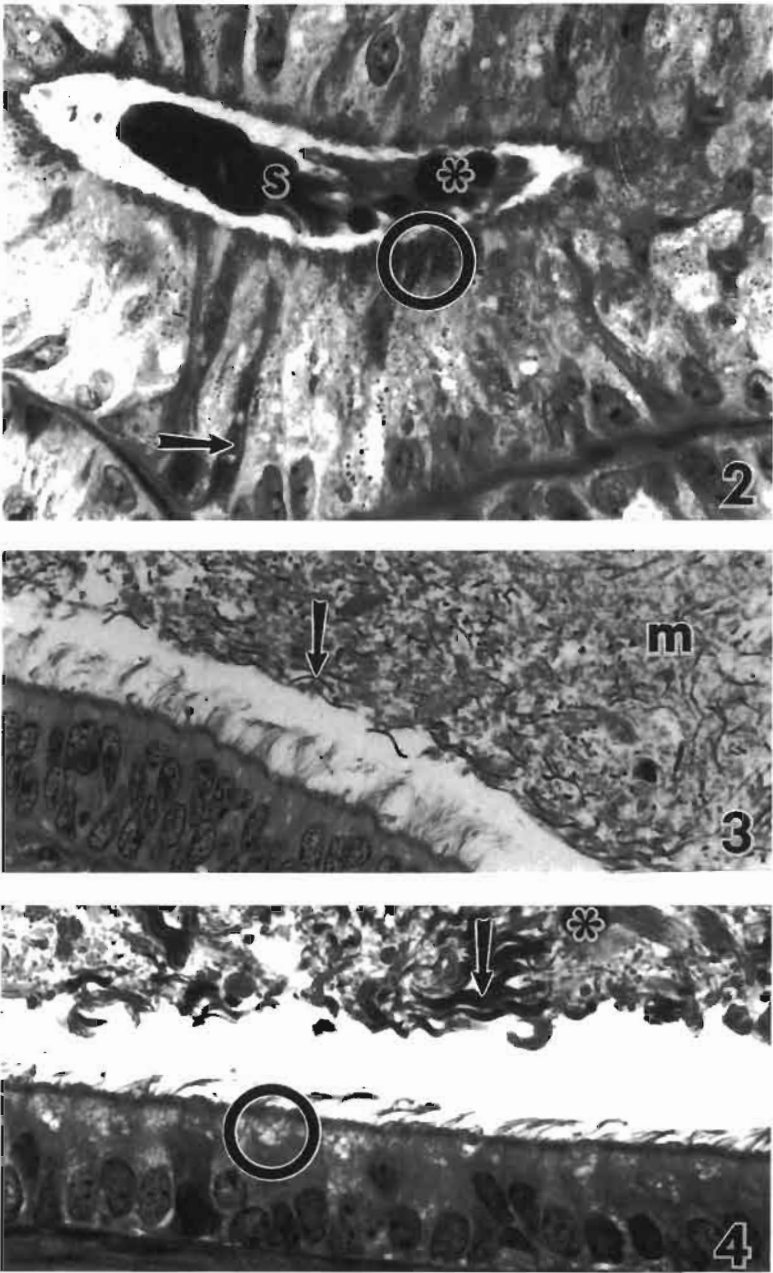


Fig. 4. - Secretory activity (circle) of the epithelium in the distal epithelium is prominent. Sperm align laterally head-to-head to form sperm aggregates (arrow). Asterisk = Leydig gland body. 600X.

the seminal vesicle. Some end blindly in the lumen while others connect to the vesicle wall to produce blind ending bays. Masses of matrix material concentrates in the lumen and associate with individual and previously aggregated sperm to produce spermatozeugmata. Spermatozeugmata enlarge as matrix masses and sperm clumps continue to associate (Fig. 7).

## DISCUSSION

Sperm are present in the seminal vesicle of elasmobranchs in various configurations, either as laterally aligned masses of sperm in an extracellular suspension, as linearly aligned clumps of sperm completely surrounded by an extracellular matrix (spermatophores) or as clumps of sperm that are surrounded by matrix but with the tails of the peripherally located sperm projecting out from the matrix into the luminal fluid of the seminal vesicle (spermatozeugmata) (Pratt and Tanaka, 1994). Edwards (1842) used the term spermatophore to describe masses of sperm surrounded by a "capsule" in cephalopods and Ballowitz (1890) suggested the term spermatozeugmata for the sperm structures in insects, in which groups of sperm are held together without being enclosed by a "capsule." Ballowitz (1895) also used the abbreviated term spermatozeugma. Spermatozoa are said to be adherent to each other by a sticky substance which permeates the spermatozeugmata. Nielsen *et al.* (1968) point out that many authors have confused spermatophores and spermatozeugmata and that most spermatophores described in fish are actually spermatozeugmata. The use of such terms as "capsule" is unfortunate. The term capsule (L. little box) is defined as a surrounding sheath or a structure in which something is enclosed and implies a separate entity such as a membrane or connective tissue investiture. The term "mass" has also been used. Mass is defined as a quantity of matter that forms a body of indefinite shape or size, usually a relatively large size. The term "aggregate" has been used and is defined as a massing of materials to form a "clump." We prefer to refer to laterally aligned sperm as "sperm aggregates" associated with an extracellular matrix. Matrix is defined in anatomy as any nonliving, intercellular substance in which living cells are embedded. Hence, extracellular matrices of various cellular origins constitute the material that is responsible for the various sperm structures seen in elasmobranchs, i.e., spermatophores and spermatozeugmata. The term "capsule" should not be used when referring to a peripheral layer of matrix.

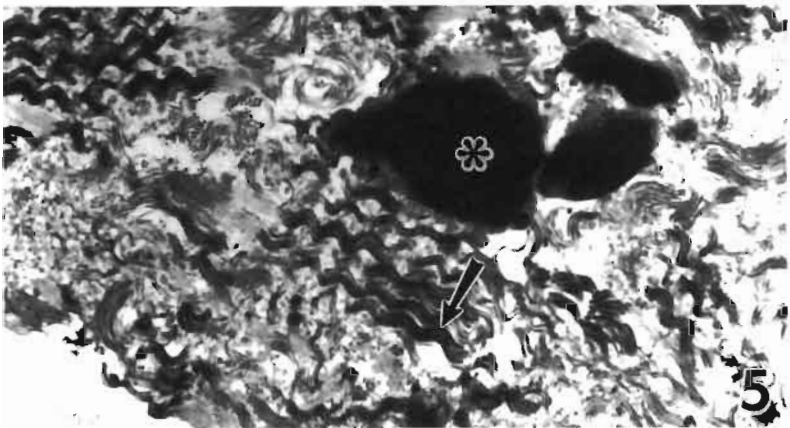
Pratt and Tanaka (1994) surveyed and described the various types of sperm associations in elasmobranchs. A "naked sperm" is an individual sperm with no surrounding matrix but this must be questioned as the luminal fluid suspension constitutes a matrix. A sperm "aggregate" is a generic term that can be applied to any cohesive mass composed of matrix and sperm, regardless of the way in which the sperm are arrayed in the matrix. Spermatophores are sperm aggregates of linearly aligned sperm surrounded by an eosinophilic matrix. The important point is that no part of the sperm projects past the perimeter of the matrix. Spermatozeugmata are fundamentally sperm aggregates in a matrix in which

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Fig. 5. - *Raja eglanteria*. The lumen of the distal epididymis contains prominent sperm aggregates (arrow) and Leydig gland bodies (asterisk). 600X.

Fig. 6. - The proximal seminal vesicle epithelium shows secretory activity (circle) and sperm aggregates (arrow). 600X.

Fig. 7. - Definitive spermatozeugmata are present in the distal seminal vesicle. Arrow = sperm heads, m = matrix. 600X



the peripheral most sperm are arrayed with their tails projecting past the perimeter of the matrix and, thereby, present a fuzzy appearance.

Spermatozeugmata have been further categorized according to the way in which sperm are arrayed and the size of the spermatozeugmata (Pratt and Tanaka, 1994): a) clump type, b) single-layered and c) compound or multilayered. Clump type spermatozeugmata occur in *Squalus acanthias* where the sperm are aligned laterally head to head with no matrix (Pratt and Tanaka, 1994) (note earlier comment on absence of a matrix). In *Hydrolagus collei* the aligned sperm combine with matrix to form spermatozeugmata in which aligned sperm occupy the center of the matrix and peripherally located sperm have their tails projecting outwardly from the matrix. It is possible that the array seen in *S. acanthias* is a transitional stage prior to or during the elaboration of matrix but before definitive spermatozeugmata as such have formed. *H. collei* may demonstrate the terminal situation in which matrix has been elaborated and have associated with previously aligned sperm.

Single layered spermatozeugmata occur in *Carcharhinus falciformis*, *C. limbatus*, *C. obscurus*, *C. plumbeus*, *C. porosus* and *Raja eglanteria* (Pratt and Tanaka, 1994). In this configuration sperm are located only at the periphery of the matrix where their tails project from the matrix.

Compound or multilayered spermatozeugmata are characteristic of *Prionace glauca*, *R. terraenovae* and *Sphyrna lewini* (Pratt and Tanaka, 1994). Peripheral clumps of sperm have their tails protruding from the matrix while inner sperm clumps form irregular projections or layers. The inner sperm have their tails pointing towards the center of the matrix.

Three main types of particulate material have been identified as occupying the male genital ducts in *Heterodontus portusjacksoni*, namely Sertoli cell bodies, Sertoli cell cytoplasts and Leydig gland bodies. Sertoli cell bodies originate in the supranuclear region of Sertoli cells just prior to spermiation. They have previously been termed Sertoli bodies (Simpson and Wardle, 1967) and problematic bodies (Holstein, 1969; Collenot and Damas, 1980). They are large, oval membrane bound, eosinophilic bodies. In *Squalus*, they have been recovered from the epididymis and cytochemical techniques reveal that their proteins are rich in lysine, cysteine and tryptophan (Pudney and Callard, 1986; Collenot and Damas, 1975). Sertoli cell cytoplasts (Pudney and Callard, 1986) have also been referred to as "small eosinophilic particles" (Jones and Jones, 1982) and "cytoplasmic bodies" (Jones *et al.*, 1984). Sertoli cell cytoplasts are remnants of Sertoli cells exclusive of the Sertoli cell bodies (Callard *et al.*, 1989). They are membrane bound structures containing mitochondria, ribosomes, lipid and endoplasmic reticulum. Since Sertoli cells may be a source of testicular steroids in elasmobranchs (Pudney and Callard, 1986; Callard *et al.*, 1989) and Sertoli cell cytoplasts contain organelles appropriate for steroid synthesis, it is possible they are responsible for the steroidogenic activity of shark semen (Simpson *et al.*, 1963; Simpson *et al.*, 1964a, 1964b). This may provide the mechanism for local control of extragonadal sperm duct activity.

Leydig gland bodies are large, eosinophilic, nonmembrane bound secretions of Leydig glands. Jones and Lin (1993) conclude that Leydig gland secretions are the main source of the increase in protein concentration of the luminal fluid in the ductus deferens in *H. portusjacksoni*.

In *H. portusjacksoni*, the efferent ductules are lined by ciliated columnar epithelium and numerous intraepithelial leukocytes. These cells may be removing particulate cellular debris from the lumen (Jones and Lin, 1993). Micropuncture studies also indicate

that there is little or no net fluid resorption in the efferent ductules (Jones *et al.*, 1984). The efferent ductules were not examined in this study.

In *H. portusjacksoni*, ultrastructural characteristics of epididymal cells suggest that their main function is protein secretion, however, micropuncture studies did not demonstrate an increase in luminal protein concentration. It is interpreted that protein associates with individual spermatozoa in the lumen immediately upon secretion (Jones *et al.*, 1984). It is possible that the sperm associated protein may play a role in sperm aggregation in the epididymis and into the ductus deferens and seminal vesicle. Net fluid transport was not detected in *Heterodontus*, yet a net resorption of sodium occurred in this region (Jones *et al.*, 1984).

The distal segment of the ductus deferens is wider than the proximal segment and contains columnar secretory epithelium with cilia and microvilli. In *H. portusjacksoni* the ciliated cells seem to be involved in heterophagic digestion, as the apical cytoplasm contains numerous vacuoles and dense bodies (Jones and Lin, 1992). Sertoli cell bodies are not present, suggesting they either disintegrate or are resorbed in the epididymis. Sertoli cell bodies were present in the ductus of *R. eglanteria*. Micropuncture studies in *H. portusjacksoni* reveal that 60% of testicular fluid is resorbed in the ductus deferens and there appears to be no net resorption of sodium from the lumen (Jones *et al.*, 1984).

The source of the matrical material is as previously detailed but other contributions cannot be ruled out, e.g., transudate from subjacent vascular beds, or other as yet undetermined sources. Changes in luminal ionic composition may also occur.

Based on the micropuncture studies of Jones *et al.* (1984), it is concluded that the luminal fluids of efferent ducts and the epididymis when compared to blood plasma showed higher levels of sodium, potassium, protein and had a greater osmolarity. Spermatocrit samples from the epididymis and ductus deferens reveals that virtually all fluid is resorbed. The per cent sperm motility increased when testicular (9%) samples were compared with epididymal samples (96%) despite any ultrastructural change. These data suggest that the epididymis and Leydig gland secretions play important roles in ion and water transport, protein secretion and maturation of spermatozoa. The nature of the sperm matrix from various segments of the male genital tract and their relation to sperm disaggregation and reaggregation is currently being analyzed (Koob and Hamlett, unpubl. data). In *H. portusjacksoni*, only the seminal vesicle has a muscular tunic, all other segments of the duct system convey sperm via ciliary activity (Jones and Lin, 1993). In *R. eglanteria*, the seminal vesicle has an inner circular and outer longitudinal tunic of smooth muscle.

The factors that mediate lateral sperm alignment without the involvement of matrix is unknown. Potential candidates include nonjunctional contacts. Cells migrating in vertebrate embryos generally do not involve organized intercellular junctional complexes. Yet the interacting plasmalemmae often come within 10-20 nm. As several known transmembrane proteins extend above the plasmalemma by 10-20 nm, two cell surface proteins could interact directly to mediate adhesion.

Substances that bind sperm clumps to matrix may include integrins. Integrins are transmembrane heterodimers. Some integrins bind to only one matrix molecule while others bind to more than one. Binding of integrins to ligands depends on extracellular divalent cations  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ , depending on the integrin. The relationship between sperm-matrix binding and sperm motility has yet to be investigated.

Spermatozoa form spherical bundles as they pass through the seminal vesicle in the Port Jackson shark (Jones and Jones, 1982; Jones *et al.*, 1984). Bundle formation has also been described in other species including *Spinax niger*, *Squalus acanthias*, *Scyliorhinus*

*rhinus stellaris*, *Chimaera monstrosa*, *Torpedo marmorata* and *T. torpedo* (Redenz and Belonschkin, 1929; Botte *et al.*, 1963).

Various suggestions have been as to why sperm aggregation in male elasmobranchs may be advantageous. Matthews (1950) suggested that spermatophores in the basking shark helped to prevent loss of semen during copulation when the semen must traverse the clasper to the female tract. A turgid spermatophore may be more hydraulically suited to passage to the female without undue loss than a fluid semen. Pratt and Tanaka (1994) mention that spermatozeugmata of carcharhinids and sphyrnids are porous and may provide a mechanism to insure adequate nourishment reaching the sperm while still packaging them for transport at copulation.

The folds found in the seminal vesicles may increase surface area for nutrient exchange and for physical support (Pratt and Tanaka, 1994). Matthews (1950) thought that the septa may play a role in spermatophore formation. He believed that epithelial secretions of the epididymis, ductus deferens and the Leydig gland mix with spermatozoa. In the upper portion of the seminal vesicle secretions form globules by action of cilia in the septal bays. The "tumbling mill" action increases the size of spermatophores. Pratt and Tanaka (1994) point out that sperm aggregates occur throughout the seminal vesicle in the short fin mako and doubt the "tumbling mill" theory is active in this species.

Sperm and the various luminal contents of the male genital ducts in *R. eglanteria* exhibit morphological changes as they progress through the various segments of the duct system. Spermatocysts are disrupted as sperm and the various other components are delivered to the efferent ducts. Sperm are released from Sertoli cells as aligned bundles associated with individual Sertoli cells. The sperm are dispersed as individual sperm in the proximal epididymis. Sperm, Sertoli bodies and Sertoli cytoplasts mingle with Leydig gland bodies in the epididymis. During transit through the epididymis, sperm begin to associate and reaggregate with their heads in lateral register. By the time the sperm arrive in the ductus deferens masses of laterally aligned sperm are present as sperm aggregates. In the seminal vesicle definitive spermatozeugmata are formed.

The role played by the matrix in which sperm are carried when the sperm is delivered to the female is unknown. Analysis of the sperm matrix is needed to ascertain its role beyond that of simply serving as a transport medium. How does the matrix and the sperm interact with the female tract and its secretions? What is the role of the male matrix in sperm activation, motility, nutrition, etc.? These and other questions remain to be answered.

**Acknowledgements.** - We wish to thank Dr. John A. Musick, Virginia Institute of Marine Sciences for providing access to specimens. This study was supported by funds provided by Indiana University School of Medicine and the Forbairt Foundation, Ireland.

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## COMPARATIVE BIOGEOGRAPHY OF THE CHONDRICHTHYAN FAUNAS OF THE TROPICAL SOUTH-EAST INDIAN AND SOUTH-WEST PACIFIC OCEANS

by

Peter R. LAST (1) & Bernard SÉRET (2)

**ABSTRACT.** - The tropical Australasian region, which includes Australia, New Caledonia, New Guinea, and the eastern sector of the Indonesian Archipelago, breaches two major ocean basins. Its complex palaeohistory is strongly reflected in the size and structure of its chondrichthyan fauna where almost a third of the world's fauna (more than 300 species) occurs. The western (Indian Ocean) and eastern sectors (Pacific Ocean) each display surprisingly high levels of intraregional endemism. Despite poorer sampling effort off Indonesia, diversity was found to be significantly greater in the Indian Ocean than in the Pacific reflecting a strong regional influence of the mega-diverse Indo-West Pacific biota. The strength of widely distributed Indian Ocean elements diminishes from west to east across the region but it is still relatively stronger off New Caledonia than the Pacific component reflecting the comparatively low biodiversity of the Pacific Plate. Tropical Australian subregions in both oceans are penetrated substantially by components from temperate areas to the south of which about half of the species are endemic to their respective oceans. Greatest biodiversity exists within demersal habitats with the continental slopes being slightly richer in species than the shelves adjacent. Slope habitats also exhibit higher levels of endemism than shallow water habitats challenging the generality of the depth-dispersal paradigm. Recent French and Australian deepwater surveys of the region have provided new insights into the composition, structure and origins of this fauna.

**RÉSUMÉ.** - Biogéographie comparée des faunes chondrichthyennes tropicales de l'Océan Indien tropical du sud-est et de l'Océan Pacifique du sud-ouest.

La région tropicale australo-asiatique, qui comprend l'Australie, la Nouvelle-Calédonie, la Nouvelle-Guinée et la partie orientale de l'archipel indonésien, met en communication deux bassins océaniques importants. Son histoire géologique complexe se reflète dans la composition de la faune chondrichthyenne qui compte presque un tiers de la faune mondiale (plus de 300 espèces). Les parties occidentale (Océan Indien) et orientale (Océan Pacifique) ont chacune de surprenants taux d'endémisme qui apparaissent aux niveaux régional et sous-régional. Malgré le manque d'échantillonnage en Indonésie, la diversité s'avère significativement plus élevée dans la région indienne que dans la région pacifique; ceci est le résultat de l'influence de la région indo-ouest-pacifique à très forte diversité biotique. L'impact des éléments distribués largement dans l'Océan Indien diminue d'ouest en est dans toute la région, mais il est encore très marqué en Nouvelle-Calédonie et plus fort que l'impact des éléments pacifiques, traduisant en cela la relativement faible biodiversité de la "plaque Pacifique". Les sous-régions tropicales de part et d'autre de l'Australie sont notablement colonisées par des éléments provenant de zones tempérées; environ la moitié de ces éléments est constituée d'espèces endémiques de leur océan respectif. La plus grande biodiversité s'observe dans les habitats benthiques, les pentes continentales étant légèrement plus diversifiées que les plateaux adjacents. Les pentes montrent parfois des taux d'endémisme plus élevés que ceux des habitats moins profonds, contredisant ainsi

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l'hypothèse habituelle de la dispersion vers les profondeurs. Les récentes campagnes exploratoires effectuées par la France et l'Australie dans cette région ont apporté de nouvelles données et conceptions sur la composition, la structure et l'origine de cette faune.

Key-words. - Chondrichthyes, ISEW, SE Indian Ocean, ISEW, SW Pacific Ocean, Biogeography.

The tropical Australasian geographic region incorporates eastern Indonesia, Australia, New Guinea and New Caledonia, south to the tropic of Capricorn (Fig. 1). The following study is an investigation of the biogeographic patterns of the chondrichthyan fishes of this region based on a comparison of the faunas of its four major subregions: eastern Indonesia (including the western Arafura, Banda and Seram Seas); northwestern Australia; northeastern Australia (including the Great Barrier Reef and adjacent plateaus and slopes of the western Coral Sea); and New Caledonia (inshore islands and steep slopes of New Caledonia and Vanuatu, and the northern Norfolk Ridge). The westerly limits of this bioregion are defined by the Wallace line in the eastern Indian Ocean. Its easterly limits are the eastern Coral Sea in the south-west Pacific. It breaches two deep oceans basins, the Pacific and Indian Oceans, which are separated from each other by the shallow Arafura Sea, and the land masses of New Guinea and continental Australia. The region has an extremely complex plate tectonic history (Oosterzee, 1997) and the bathymetry and oceanography of the contemporary environment is equally complex. Its sea floor is heavily textured, its habitat diversity is high, and its marine fauna is one of the richest on earth with a high level of endemism.

The chondrichthyan fauna of the entire region is poorly documented, although those of the Australian subregions are best described (e.g., Whitley, 1940; Last and Stevens, 1994). Much of the fauna is undescribed and revisionary studies by the authors and several colleagues (i.e., Carvalho, Compagno, McEachran, Stehmann, Stevens, Yearsley) are in progress. Knowledge of its inshore communities are attributed largely to the efforts of 19th and early 20th C naturalists (e.g., Bleeker, 1852; Annandale, 1909) and collectors (e.g., McCulloch, 1929; Fowler, 1941) whereas information on the deepwater component has been acquired more recently from trawl surveys, mostly in search of new fishery resources (e.g., Gloerfelt-Tarp and Kailola, 1984; Sainsbury *et al.*, 1985; Williams *et al.*, 1996). These include the cruises of the fishery research vessels «Bawal Putih 2», «Jurong», «Karubar» (eastern Indonesia), «Courageous», «Soela» and «Southern Surveyor» (northern Australia), and «Alis», «Capricorne», and «Vauban» (eastern Coral Sea), which within the last two decades, have variably surveyed continental shelves and slopes of the region to depths of about 1000 m. Much of the data from these cruises is yet to be published but initial studies of the deepwater communities have identified high levels of diversity and sibling speciation (Séret and Last, unpubl.)

The following study describes and assesses key structural features of this fauna such as its relative size, levels of endemism and faunal similarity, and identifies biogeographic patterns across the region. Preliminary hypotheses are proposed to explain the likely origin and derivation of this fauna.

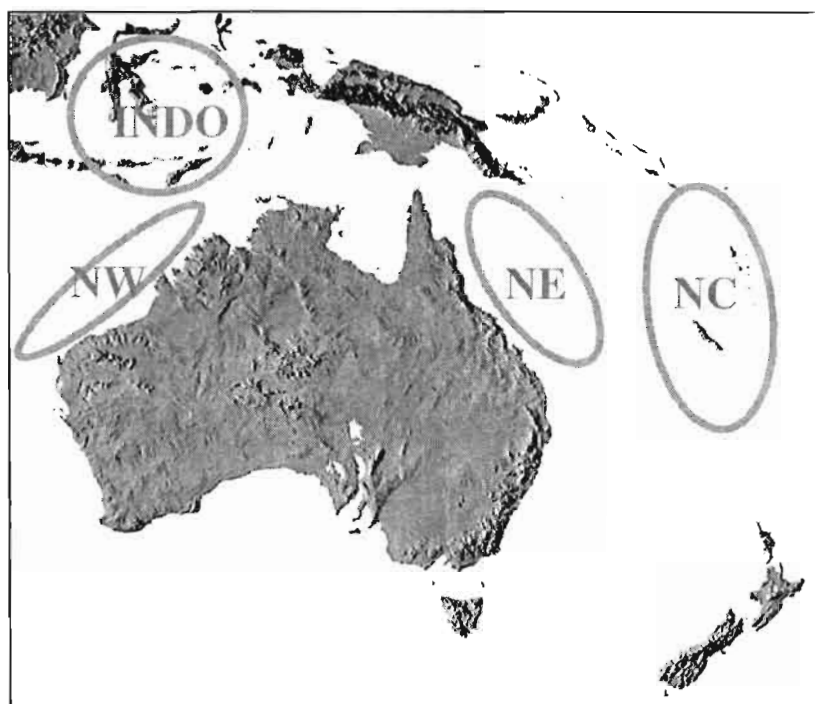


Fig. 1. - Australasian region. INDO: Indonesian subregion; NC: New Caledonian subregion; NE: north-eastern Australian subregion; NW: northwestern Australian subregion.

## METHODS

Species lists for each subregion were compiled from multiple sources including a large body of unpublished data held by the authors which is being summarised in a separate document (Séret and Last, in prep.). Species were classified into one of the distributional groups listed below based on information from the published literature (e.g., Chen, 1963; Masuda *et al.*, 1984; Monkolprasit, 1984; Compagno 1984, 1988; Compagno *et al.*, 1989) as well as several key regional references that are currently in press such as sharks of the South China Sea (Compagno, in press), rays of the South China Sea (Last and Compagno, in press), and FAO identification sheets to sharks and rays of the western Central Pacific (Compagno *et al.*, in press). The Australasian fauna consists of 5 primary biogeographic components and 23 secondary elements: 1. Subregional endemics (5 elements), endemic species confined to each of the four subregions or New Guinea; 2. Extraregional temperate component (2 elements), New Zealand species, or Australian temperate species that either have a primary distribution in the southwestern (Flindersian) or southeastern (Peronian) Australian Provinces, or have a widespread distribution around the southern coast (*sensu* Whitley, 1932; Thackway and Cresswell, 1997); 3. Australasian regional component (3 elements), Coral Sea species (Australian/New Caledonian endemics or species confined to the south-west Pacific), east Indian Ocean species (confined to eastern Indonesia and tropical Australia), and tropical Australasian species (species occurring only off tropical Australia or extending more broadly across

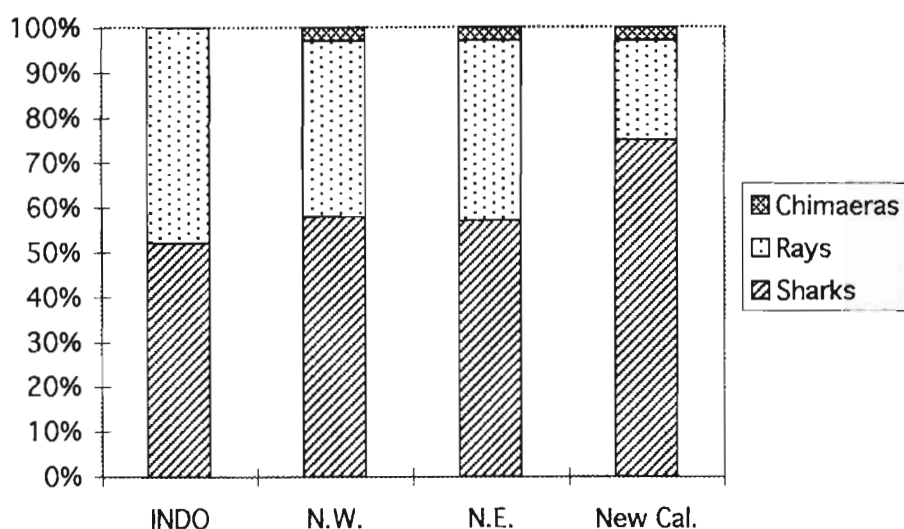


Fig. 2. - Proportional composition of primary chondrichthyan groups by region. INDO: Indonesian subregion; NC: New Caledonian subregion; NE: northeastern Australian subregion; NW: northwestern Australian subregion.

the Australasian region); 4. Indo-Pacific component (8 elements), widespread Pacific species, Indo-Malay endemics, Indo-NW Pacific species (Asian species not penetrating Australian seas or westward beyond the Indo-Malay Archipelago), Indian Ocean species (in the Indo-Malay Archipelago but not found off Australia or well into the North Pacific), western Indo-Australian species (wide-ranging in the Indian Ocean to Australia but with minimal penetration of the NW Pacific), northern Indo-Australian species (wide-ranging in the NW Pacific to Australia but with minimal penetration of the Indian Ocean), widespread temperate Indo-Pacific species (distribution centred in band across temperate Indo-Pacific), and widespread tropical Indo-Pacific species (distributed widely across the tropical Indo-Pacific); 5. Ubiquitous component (5 elements), extremely wide-ranging species that have anti-tropical, circumtropical, and cosmopolitan distributions, or with species found in the Indo-Atlantic or Pacific-Atlantic basins. Intra-regional faunal similarity was investigated using Sorensen's coefficient (*sensu* Pielou, 1979).

## RESULTS

### The regional fauna

#### Size and structure

Some 313 or so chondrichthyan species, almost a third of the world's fauna, occur in this region. However, this figure is likely to be conservative. The taxonomy of some groups remains unresolved and the region has not been fully explored. We expect the number of species known from the region to increase significantly when remote areas, such as the slopes and basins of eastern Indonesia, have been surveyed and all available material has been examined. Nevertheless, current information is considered to be adequate to gain an understanding of the major faunal patterns within the region.

Of the faunas of the four subregions, the northwestern Australian assemblage (178 species) is the most species rich and the New Caledonian assemblage least diverse (92 species) (Fig. 2). The relative proportion of sharks, rays and chimaeras was identical in the two Australian subregions despite being in different oceans and differing slightly in species richness. Sharks are about 20% more diverse than rays off tropical Australia but exhibit an even greater relative dominance off New Caledonia (almost three-quarters of the species). The absence of several bottom-dwelling shark groups (e.g., hemiscylliids, heterodontids, squatinids or pristiophorids) and lower ray diversity of the New Caledonian subregion may be related to the lack of suitable habitat rather than patterns of evolution. In contrast, the numbers of sharks and rays in the eastern Indonesian subregion are about equal. Here the greater relative diversity of rays and benthic sharks may be due to higher inshore habitat diversity and more pronounced influence of the large Asian fish fauna. The absence of chimaeras in the eastern Indonesian assemblage is likely to reflect the inadequacy of deep sea survey coverage of the subregion.

The ordinal level structures of the eastern and western tropical Australian shark faunas are also extremely similar despite there being only 60% overlap in species composition (Table I). About half of shark species in the northwestern Australia, northeastern Australia and New Caledonian subregions belong to the order Carcharhiniformes, of which the whaler sharks (carcharhinids) are the dominant group. In the Indonesian subregion, almost three-quarters of the species belong to this order and a massive 43% of them are whaler sharks. Once again, fewer dogfishes in eastern Indonesia may be linked to the relative paucity of material from deepwater. In comparison, the continental slope chondrichthyans of New Caledonia are better known. Here, the dominance of dogfishes (33%) and the absence of the shark orders Pristiophoriformes, Squatiniformes, and Heterodontiformes and the ray order Pristiformes off New Caledonia may be habitat related.

The order Myliobatiformes, which includes the eagle and stingrays, is the dominant ray group throughout the region (Table II). Once again, the northwestern Australian and northeastern Australian assemblages are similar in their ordinal level structure but the greater relative importance of urolophids to dasyatids in the east is likely to be an example of ecological replacement. Stingrays are relatively more diverse in both western subregions (30-31% of Indian species versus 25-26% of Pacific species) whereas stinga-

Table I. - Proportional species richness of shark groups within each subregion.

	E. Indo.	NW Aust.	N.E. Aust.	New Cal.
Order	(%)	(%)	(%)	(%)
Hexanchiformes	2	3	3	4
Squaliformes	8	21	17	33
Pristiophoriformes			1	
Squatiniformes		2	1	
Heterodontiformes	2	2	2	
Orectolobiformes	11	12	14	4
Lamniformes	6	9	8	9
Carcharhiniformes	71	51	53	49
Fam. Carcharhinidae	43	27	28	19
Fam. Scyliorhinidae	17	13	13	20
Other families	11	11	13	10
Total species	63	105	96	69

rees are relatively more diverse in the eastern subregions (13-21% of Pacific species versus 3-7% of Indian species). These observations are supported by their relative levels of abundance in catches (Last, unpubl. data). Of the other ray groups, skates were the next most diverse group comprising about a fifth of the ray species found off tropical Australia. Once again, smaller skate assemblages off New Caledonia are likely to be related to a paucity of sedimentary habitats and off eastern Indonesia to the lower levels of deepwater exploration. The proportion of shovelnose rays (10-12%) was remarkably similar across the region.

#### *Faunal similarity*

The species compositions in each of the subregions differs substantially from each other. Also, the relative levels of faunal overlap of the eastern Indonesian assemblage diminish from west to east (Table III). However, the nearby northwestern Australian assemblage is more similar in composition to the northeastern Australian assemblage (sharing about 62% of its species) rather than the eastern Indonesian assemblage (sharing about 43% of its species). Similarly, the northeastern Australian assemblage exhibited much greater relative overlap with the northwestern Australian assemblage than with the neighbouring New Caledonian assemblage. Relative similarity between the New Caledonian assemblage to the assemblages of the other subregions (19-22%) is due both to its uniqueness and its lower relative diversity. Only about half of the New Caledonian species were found off Australia. The strong similarity of the two Australian assemblages is due to the presence of a pronounced widespread Australian element in the fauna.

#### *Biomic diversity*

The representation of chondrichthyan diversity within primary aquatic ecosystem complexes (or biomes) is extremely similar off eastern and western Australia (Table IV).

Table II. - Proportional species richness of ray groups within each subregion.

	E. Indo.	N.W. Aust.	N.E. Aust.	New cal.
Order	(%)	(%)	(%)	(%)
Pristiiformes	8	7	6	
Rhinobatiformes	12	10	10	11
Torpediniformes	3	9	8	5
Rajiformes	17	21	21	16
Myliobatiformes	60	52	56	68
Fam. Dasyatidae	30	31	25	26
Fam. Urolophidae	3	7	13	21
Other families	27	13	17	21
Total species	60	67	63	19

Table III. - Sorensen's indices of faunal similarity between subregions.

Subregion	E. Indo.	N.W. Aust.	N.E. Aust.	New cal.
East Indonesia	1			
N.W. Australia	0,51	1		
N.E. Australia	0,42	0,64	1	
New Caledonia	0,31	0,33	0,38	1



The biomic structures of these subregions are more alike than structures observed within subregional pairs from the same ocean basin. Within those subregions having the greatest variety of inshore habitats (i.e., eastern Indonesia, northwestern Australia, northeastern Australia) the shelf assemblages (61-70% of species) were more species rich than those offshore. However, off New Caledonia most species live demersally on the continental slope with only 30% of species identified as belonging to a shelf assemblage. The freshwater/estuarine assemblage is absent in New Caledonia and tends to be small generally throughout the region (uniformly 2% elsewhere). Meso/bathypelagic communities of the eastern Indonesian subregion have not been well surveyed so this assemblage remains unknown.

### Biogeographic patterns

#### *Regional structure*

Strong biogeographic patterns emerge from a comparison of the four subregions based on the relative influence of the 7 major distributional components (Table V). A half or slightly less of the Australian and New Caledonian chondrichthyans, but only about a quarter of those from the eastern Indonesian subregion, are confined to the Australasian region. Once again, the faunas of two Australian subregions were very similar to each other in structure and quite different from the subregions adjacent. Levels of intraregional endemism are reasonably high (12-18% in eastern Indonesian, northwestern Australian, and northeastern Australian subregions) and very high off New Caledonia (32%). Endemism is almost exclusively of demersal species (Table VI). It is most pronounced on

Table IV. - Proportional species richness for primary biomes within each subregion.

Biome	E. Indo. (%)	N.W. Aust. (%)	N.E. Aust. (%)	New Cal. (%)
Estuarine/freshwater	2	2	2	
Shelf-demersal	43	44	41	15
Shelf-pelagic	25	15	18	15
Slope-demersal	25	30	32	57
Epipelagic	5	5	6	8
Meso/bathypelagic		4	1	5
Total species	123	178	164	92

Table V. - Biogeographic structure of tropical Australasian chondrichthyans.

Component	E. Indo. (%)	N.W. Aust. (%)	N.E. Aust. (%)	New Cal. (%)
Subregional endemic	12	15	18	32
Extraregional temperate		12	15	3
East Indian Ocean	7	4		
Coral Sea			3	5
Tropical Australasian	2	14	14	2
Indo-Pacific	59	32	27	25
Ubiquitous	20	23	23	33
Total species	123	178	164	92

the continental slope (rather than inshore) in all subregions except northwestern Australia where more than 60% of the endemics are demersal shelf species.

The northern incursions of Australian temperates are significant along both Australian seabords (12-15%) and differ markedly in species composition in each ocean. The bulk of these species are temperate endemics from either the south-east (Peronian) or the south-west (Flindersian) Australian faunal provinces that stray into adjacent tropical waters. The New Caledonian subregion is penetrated by a small temperate element from New Zealand but no Australian species. No Australian temperate chondrichthyans occur in the seas of the eastern Indonesian subregion. A small east Indian element (4-7%) was identified along with an equivalent small Coral Sea element (3-5%). A significant tropical Australian element (14% off each seaboard) is rudimentary within the eastern Indonesian and New Caledonian subregions (2%).

The two major groups of widespread elements include those species with an Indo-Pacific distribution and an assemblage of ubiquitous species. The eastern Indonesian fauna is dominated by a large assemblage (almost 60%) of species that also occur more widely throughout the Indo-Pacific. This element is also prominent in the other subregions but declines slightly from west to east. It is important to note that this element is more pronounced than any equivalent Pacific element which is consistent with a regional fauna whose primary origins are Indo-West Pacific based rather than derived in the Pacific Ocean. Most of the ubiquitous species were found across the region and these consisted of between a third and a fifth of the species in each subregion.

#### *Indo-Pacific component*

Careful examination of the substructure of the Indo-Pacific component provides insight to the likely biogeographic history of the region. The total number of Indo-Pacific species (including those with both Pacific and Indian Ocean distributions) also declines from west to east (Table VII). Similarly, the configuration of the Indo-Pacific components are remarkably similar off eastern and western Australia despite them being in different oceans. Also, the substructure off New Caledonia is more similar to Australian shores than to eastern Indonesia. It appears as if the Australian-New Caledonian region has been penetrated from the west by a strong but relatively uniform Indo-Pacific assemblage.

A large tropical Asian assemblage, consisting of species found only in the Indo-Malay Archipelago (3%), westward into the central Indian Ocean (26%), or northward to Japan (11%) penetrates eastern Indonesia but none of its species reaches further eastward to Australia or New Caledonia. The combined proportion of Indian Ocean based Indo-West Pacific species off eastern Indonesia is 44% versus 39% for those that extend into the north-west Pacific. Consequently, the faunal structure in the region to the east of Indone-

Table VI. - Distribution of subregional endemics in environments.

Environment	E. Indo.	N.W. Aust.	N.E. Aust.	New Cal.
<b>Demersal</b>	15 (100%)	26 (100%)	27 (93%)	28 (97%)
Shelf-demersal	2 (13%)	16 (62%)	7 (24%)	1 (3%)
Slope-demersal	13 (87%)	10 (38%)	22 (76%)	28 (97%)
<b>Pelagic</b>			2 (7%)	1 (3%)
<b>Total species</b>	15	26	29	29

sia is also likely to be dominated by Indian Ocean elements. Instead, these assemblages are dominated by Indo-West Pacific species (44-48%) that extend from the south-west Pacific to well north off south-east Asia but which do not penetrate far into the Indian Ocean. A much smaller suite of species (23%) extends from the south-west Pacific well into the Indian Ocean but not far into the north-west Pacific.

The relative strength of the widespread Indo-Pacific element increases within the region from west to east due to a decline in strength of more restricted Indo-Pacifics from west to east. However, the number of widespread Indo-Pacifics, varying between 10-14 species, is reasonably even across the region. In comparison, the widespread Pacific element is very small with 9% of the New Caledonian species, represented as trace elements (2-4%) off Australia, and absent from the eastern Indonesian subregion.

#### *Ubiquitous component*

The ubiquitous component, which consists of 25-41 species across the region, is dominated by cosmopolitan elements (comprised of 49-60% species) (Table VIII). Once again, these species are more closely linked to the Indian Ocean than to the Pacific Ocean. Only 3-8% of the ubiquitous Australian and New Caledonian species have a Pacific-

Table VII. - Biogeographic structure of the Indo-Pacific chondrichthyan components.

Element	E. Indo. (%)	N.W. Aust. (%)	N.E. Aust. (%)	New Cal. (%)
<b>Widespread Pacific</b>		4	2	9
<b>Indo-West Pacific (to Indonesia)</b>				
Indo-Malay endemic	3			
Indo-NW Pacific	11			
Indian Ocean	26			
<b>Indo-West Pacific (to Australia)</b>				
northern Indo-Australian	28	48	44	27
western Indo-Australian	18	23	23	9
<b>Widespread Indo-Pacific</b>				
temperate element	3	9	11	18
tropical element	11	16	20	37
<b>Total species</b>	71	56	44	23

Table VIII. - Biogeographic structure of the ubiquitous chondrichthyan components.

Element	E. Indo. (%)	N.W. Aust. (%)	N.E. Aust. (%)	New Cal. (%)
<b>Indo-Atlantic</b>	28	24	18	10
<b>Pacific-Atlantic</b>		5	8	3
<b>Antitropical</b>		2		7
<b>Circumtropical</b>	20	20	24	20
<b>Cosmopolitan</b>	52	49	50	60
<b>Total species</b>	25	41	38	30

Atlantic distribution whereas 10-18% of the Indo-Atlantic species occur in the Pacific subregions. Pacific-Atlantic species are absent from the eastern Indonesian subregion but 28% of its ubiquitous component is found throughout the Indo-Atlantic. The distribution of these Indo-Atlantic species follows the pattern exhibited by more restricted Indo-Pacific in decreasing in richness from west to east across the region. The anti-tropical element is small to non-existent but the circumtropical element is well defined and consistent within all subregions (20-24%).

### Faunal origins

Wilson and Allen (1987) have attempted to provide an explanation for the origin of Australia's fish fauna. The modern Indian and western Pacific Oceans are considered remnants of a once larger Tethys Sea (Oosterzee, 1997). A pan-tropical Tethyan fauna is thought to have dominated northern Australasian seas since the Tertiary. Some of these Indo-Pacific groups were able to radiate across the Tethyan Sea to colonise the broader region as well as the precursor of the Atlantic Ocean. These elements are evident within the chondrichthyan fauna as widespread components. The Tethyan fauna speciated greatly during the formation of shallow basins (i.e., Indo-Malay Archipelago) created by the partial separation of these oceans when the Australian shield impinged upon the Asian Plate. Most of the ancestral forms of the present fauna are presumed to have been derived before and during this period of fragmentation in the early Tertiary. Westward circulation between the Pacific and Indian Oceans is thought to have virtually ceased but biological dispersal into the Tethyan basins persisted providing the evolutionary ingredients for the modern marine megabiota, the richest of any region (Briggs, 1974). Chondrichthyan species diversity is significantly greater in the Indian Ocean than the Pacific despite poorer sampling efforts off Indonesia. Similarly, the influence of Indian Ocean elements in the Australasian region is substantially greater than that of Pacific elements.

The extant chondrichthyan fauna of the region is the product of several periods of faunal mixing and isolation. The deepwater fauna occurring along the most likely contemporary tropical dispersal route (i.e., along the northern coast of New Guinea) is almost unknown so we are unable to even speculate as to its affinities. Also, this pathway is likely to have changed greatly since the existence of a Tethyan Sea as islands to Australia's north are geological composites formed by plate tectonic rifts and collisions (Parenti, 1991). However, given that many extant species occur only in deepwater and cannot pass through the shallow aperture between northern Australia and New Guinea, this pathway is likely to be important for dispersal and is the most plausible corridor for the high proportion of north-west Pacific species occurring in the Coral Sea. Continuous deepwater around the southern coast of Australia links both ocean basins but the contemporary temperate fauna is strikingly different in composition to those of the north (Last and Stevens, 1994) and is unlikely to have been an important Quaternary pathway. Continental shelf chondrichthyans are likely to be less constrained in recent times by an east-west intraregional barrier. The shallow water components were able to coalesce with the opening of Torres Strait during the flooding of the shelf area between Australia and New Guinea in the Pleistocene (Wilson and Allen, 1987).

Some evidence exists to suggest that the regional was penetrated by elements from the Indian and north-west Pacific during separate events and at differing levels of impact. The absence off Australia of suites of Indian Ocean genera and species found off eastern Indonesia (e.g., *Chaenogaleus*, *Scoliodon*, and *Lamniopsis*) is suggestive of either a speciation event with a barrier between most of Australia and Indonesia, or of an Indian

Ocean origin with the new assemblage unable to subsequently disperse east across the region. Either way the southern pathway from the Indian Ocean appears to have been interrupted at some stage.

Similarly, the deep trenches and seas that separate parts of the Australasian region are likely to have acted as historical barriers that have affected the composition of the extant fauna. The high respective levels of intraregional endemism are indicative of lengthy isolation periods of at least some of these elements. Some benthic chondrichthyans (e.g., skates and stingarees), whose distributions are heavily constrained by depth related barriers (such as the deep ocean trenches and basins), are good biogeographic indicators. For example, islands of New Caledonia and New Zealand are partially connected across the deep south-west Pacific rim via the Norfolk Ridge. However, despite these submarine ridges acting as the likely pathways for the dispersal of several eurybathic teleost groups and some chondrichthyans (e.g., *Mustelus* and *Squalus*) no stingarees occur off New Zealand. Pavorajine skates species appear to have been isolated similarly in the Coral Sea.

Knowledge of the biogeographic structure and faunal origins within any region is only a good as the groups selected for its appraisal. When available, informative groups should be used to interpret biogeographic patterns rather than uninformative groups. The most informative groups tend to be genera or families that are medium to highly speciose and whose members mostly have restricted ranges. Small groups with predominantly wide-ranging species are typically less informative. Some sharks and rays, by the nature of their life history (e.g., low fecundity, narrow home range, short breeding period and poor juvenile dispersal) can be used as indicators to determine biogeographic patterns within realms. Possibly the most useful groups in this region include stingarees (*Urolophus* and *Trygonoptera*), stingrays (*Dasyatis* and *Himantura*) skates (*Anacanthobatus*, *Dipturus*, *Irolita* and *Pavoraja*), catsharks (*Asymbolus*, *Cephaloscyllium* and *Galeus*) and shovelnose rays (*Aptychotrema* and *Rhinobatos*). The family Urolophidae, which has mostly narrow-ranging species, occurs off both Australian coasts, New Caledonia, and along the northern New Guinea pathway to Japan. Based on centre of origin theory (Pielou, 1979) and the high level of speciation in the Australian region (28+ species versus only 1 species west of Australasia), it is most likely that the evolutionary pathway of this group extended from Australia rather than to it from the North Pacific. A similar argument probably can be applied to several other groups (e.g., *Pristiophorus*, orectolobids, *Cephaloscyllium*, and skates of the *Notoraja* complex).

An important feature of the Australian fauna is its structural similarity (based on the levels of representation of the three main chondrichthyan groups, their orders, their biomic structure, levels of endemism, and similarity to the more widely ranging Indo-Pacific fauna) despite sharing only about two-thirds of its species across oceans. These subfaunas are more similar to each other than they are to those of nearby regions in the same ocean. Interestingly, 11 genera (comprising 62% of the endemic species) of 30 regional endemic genera occur in both Australian subregions. The most diverse genera include *Squalus*, *Narcine*, *Pavoraja*, *Dipturus*, and *Urolophus*. The high level of sibling speciation across the region is indicative of one or more vicariance events, which based on the groups involved, may have occurred after the formation of barriers across both northern and southern Australian distributional pathways. The pathways would seem at least partially disjunct at their northern extremities as there seems to be limited spill over into adjacent subregions within the same ocean. In the south, vicariance speciation is thought to be due largely to rises and fall in sea level in the Pleistocene (Wilson and Al-

len, 1987). Species within some groups, such as *Trygonoptera*, *Aptychotrema*, and probably *Asymbolus* and *Pavoraja*, are likely to have arisen this way. These groups, which are most diverse in temperate seas, have unique sister species off either side of tropical Australia that are likely to have dispersed into these regions from the south. Similarly there are groups that are likely to be linked to a northern pathway (e.g., *Atelomycterus*, *Narcine*, *Insentiraja*, and *Anacanthobatis*). The association of the faunas of both ocean basins, presumably via a northern pathway in recent times is probably best demonstrated through the genus *Galeus*. Sibling species, *G. gracilis* (Indian Ocean) and *Galeus* sp. (Pacific Ocean) are so similar in morphology that the females are virtually indistinguishable. However, the external clasper morphologies of mature males differ so greatly from each other that they could be considered to be generically distinct.

The uniform infraregional structure of the contemporary Australian fauna at the supraspecific level means that similar ecomorphotypes will be represented off each region despite differences in the species mix. However, some differences in structure found in adjacent subregions may be attributed to habitat availability. New Caledonia is comparatively simple in habitat diversity inshore and this is reflected in the structure of its chondrichthyan fauna. Several soft-bottom groups are absent from this subregion. Conversely, the fauna of eastern Indonesia is relatively richer in soft-bottom elements found in south-east Asia. These habitats are particularly diverse across the Indo-Malay Archipelago. Wilson and Allen (1987) explained differences in the relative dominance of inshore continental to coral reef fishes between tropical oceans off Australia as an example. Continental fishes are dominant off northwestern Australia (where coral reef habitat is comparatively limited) and coral reef fishes are more diverse off eastern Australia (where coral reef habitats are more diverse).

In summary, the chondrichthyan fauna of the region is likely to be derived primarily of ancestral groups that evolved in the Indo-West Pacific but which are penetrated by derivatives of an older widespread Tethyan fauna and more recent post Gondwanan elements from temperate southern Australia. The influence of central and eastern Pacific elements is minimal. Habitat availability and diversity is a likely determinant of contemporary composition within the region. This research also highlights the need for exploration of remote zones such as the New Guinean slope and parts of Indonesia to obtain a better picture of the biodiversity of the region.

**Acknowledgements.** - The authors wish to thank the many field scientists and fishermen that have contributed survey material without which this study would not have been possible.

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## SPECIES GROUPING WITHIN THE GENUS *APRISTURUS* (ELASMOBRANCHII: SCYLIORHINIDAE)

by

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**ABSTRACT.** - Thirty two species are currently recognized in the genus *Apristurus*, which is one of the most taxonomically confusing taxon among sharks. Based on the examination of nearly four hundred specimens from the Pacific, Indian and Atlantic oceans, the species are divided into three species groups. The first group (*longicephalus* group), consisting of *Apristurus herklotsi* and *A. longicephalus*, is characterized by an extremely long snout. Remaining 30 species with a short snout are grouped into *brunneus* and *spongiceps* groups. The species of the *brunneus* group have higher spiral valve counts (14-22), longer upper labial furrows than the lower ones, and discontinuous supraorbital sensory canals. The species of the *spongiceps* group have lower spiral valve counts (7-12), upper labial furrows subequal to or shorter than the lower ones, and continuous supraorbital canals. The former group contains 20 species *Apristurus acanutus*, *A. atlanticus*, *A. brunneus*, *A. canutus*, *A. gibbosus*, *A. indicus*, *A. internatus*, *A. investigatoris*, *A. japonicus*, *A. laurussonii*, *A. macrorhynchus*, *A. macrostomus*, *A. micropterygeus*, *A. nasutus*, *A. parvipinnis*, *A. platyrhynchus*, *A. saldanha*, *A. sibogae*, *A. sinensis* and *A. verweyi*. The latter group consists of 10 species *Apristurus aphyodes*, *A. fedorovi*, *A. kampae*, *A. manis*, *A. microps*, *A. pinguis*, *A. profundorum*, *A. riveri*, *A. spongiceps* and *A. stenseni*.

**RÉSUMÉ.** - Groupements d'espèces dans le genre *Apristurus* (Elasmobranchii, Scyliorhinidae).

Trente deux espèces sont actuellement reconnues dans le genre *Apristurus*, qui est l'un des taxa les plus confus chez les requins. Une étude basée sur l'examen de près de 400 spécimens provenant des océans Pacifique, Indien et Atlantique, a permis de séparer ces espèces en trois groupes. Le premier (groupe-*longicephalus*) inclut *Apristurus herklotsi* et *A. longicephalus*, et se caractérise par un museau extrêmement long. Les 30 autres espèces avec un museau court se séparent en deux groupes: le groupe-*brunneus* et le groupe-*spongiceps*. Les espèces du groupe-*brunneus* ont un nombre élevé de spires à la valvule spirale (14-22), des sillons labiaux supérieurs plus longs que les inférieurs, et des canaux sensoriels supraorbitaires discontinus. Les espèces du groupe-*spongiceps* ont un nombre moins élevé de spires à la valvule (7-12), des sillons labiaux supérieurs aussi longs ou bien plus courts que les inférieurs, et des canaux sensoriels supraorbitaires continus. Le premier groupe comprend 20 espèces: *Apristurus acanutus*, *A. atlanticus*, *A. brunneus*, *A. canutus*, *A. gibbosus*, *A. indicus*, *A. internatus*, *A. investigatoris*, *A. japonicus*, *A. laurussonii*, *A. macrorhynchus*, *A. macrostomus*, *A. micropterygeus*, *A. nasutus*, *A. parvipinnis*, *A. platyrhynchus*, *A. saldanha*, *A. sibogae*, *A. sinensis* et *A. verweyi*. Le deuxième groupe comprend 10 espèces: *Apristurus aphyodes*, *A. fedorovi*, *A. kampae*, *A. manis*, *A. microps*, *A. pinguis*, *A. profundorum*, *A. riveri*, *A. spongiceps* et *A. stenseni*.

**Key-words.** - Scyliorhinidae, *Apristurus*, Spiral valves, Labial furrows, Sensory canal, Grouping, Taxonomy.

Sharks of the genus *Apristurus* are a group of deep water catsharks that mainly inhabit continental slopes and submarine ridges below 500 m. They are characterized by a

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large anal fin, which is separated from the lower caudal fin lobe by only a notch, a flat snout and absence of distinct crest-like dermal denticles along the upper margin of the caudal fin.

Taxonomy of this genus has been studied by Springer (1966, 1979), Nakaya (1975), Cadenat and Blache (1981), and Compagno (1984). Since then, many species have been described (Zhu *et al.*, 1981, 1986; Deng *et al.*, 1983, 1985, 1988; Dolganov, 1985; Meng *et al.*, 1985). As a result of these studies, the number of nominal species reached 37 by 1988. However, as noted by Compagno (1988), none of the studies critically compared the species to assess the number of valid taxa, and he included some undescribed species in his list of *Apristurus*, suggesting the presence of further more undescribed species. Paulin *et al.* (1989) listed five species from New Zealand, but they did not name them. Nakaya (1991) reviewed the long snouted *Apristurus* and synonymized five nominal species with *A. herklotsi*. Last and Stevens (1994) recognized eight species from Australia, but could identify only *A. longicephalus*, leaving the other seven species as *Apristurus* spp. Recently, Nakaya and Stehmann (1998) described *A. aphyodes* from eastern North Atlantic, but Nakaya and Sato (1998) placed *A. maderensis* under synonymy of *A. laurussonii*. According to B. Séret (MNHN, Paris, pers. comm.), there are also several unknown species in the waters of New Caledonia. Some of these species from the south Pacific and Australia may be conspecific each other, but some of them are apparently undescribed species. To summarize number of the species in the genus, 32 species are currently recognized as distinct, but the number will increase by the description of those unnamed species.

The genus *Apristurus* is now one of the most confusing groups among sharks, and its taxonomy is getting more difficult with increase of the number of species. In fact, the identification is almost impossible in some areas, as mentioned above. Compagno (1988) proposed ten species groups in the genus *Apristurus*, based on a combination of various characters. For the actual taxonomic purposes, it is quite helpful to subdivide the genus into some species groups, but Compagno's groups are not clearly separable because most of the characters he used are subjective, or largely overlapping. Here, we made a morphological investigation of the entire genus, and we recognized three distinct species groups in the genus by some objective characters.

## MATERIALS AND METHODS

A total of 413 specimens, representing all the 38 nominal species of the genus, were studied, and they are given below in the list of specimens examined. The type specimens of 33 species were examined, but those of *A. saldanha*, *A. microps*, *A. nasutus* and *A. platyrhynchus* were unavailable, because they are missing. We could not get access to the type specimens of *A. fedorovi*. Identification of *A. microps* and *A. saldanha* follows Compagno (1984) and Bass *et al.* (1975), respectively. Method of measurements follows Nakaya (1991). Institutional acronyms follow Leviton *et al.* (1985) unless otherwise noted.

## RESULTS

We examined various morphological characters, and the following four characters were found to be consistent and useful in objectively distinguishing the groups within the genus *Apristurus*.

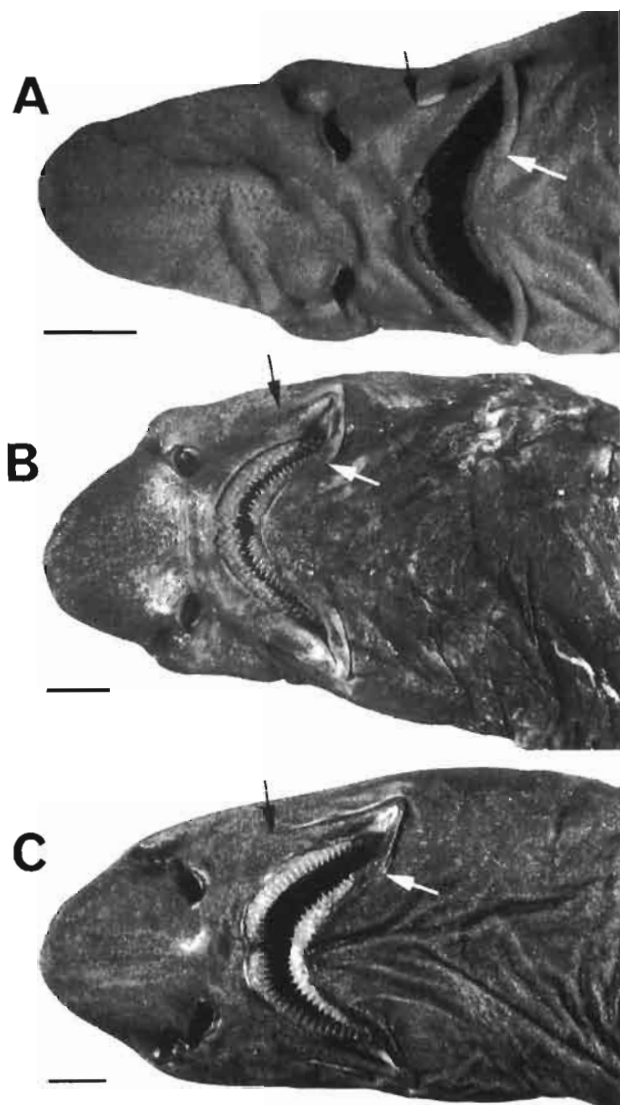


Fig. 1. - Three morphs of *Apristurus*. A: *Apristurus herklotsi*, BSKU 23110, female, 316 mm TL; B: *A. fedorovi*, HUMZ 72871, male, 649 mm TL; C: *A. brunneus*, HUMZ 105610, male, 609 mm TL. Black and white arrows indicate beginning points of upper and lower labial furrows, respectively. Scale bars = 10 mm.

### Snout length

As shown in figure 1, the length of snout before nostril is quite different among species. For example, *Apristurus herklotsi* (Fig. 1A) has unusually long snout before nostril, while the snout is short in *A. fedorovi* (Fig. 1B) and *A. brunneus* (Fig. 1C). Figure 2 shows the relationships between snout length before nostril and interorbital

width in the all species of the genus, and this clearly indicates presence of two different groups, i.e., one (solid circles) with a longer prenostril snout length than interorbital width and the other (open circles) with a shorter prenostril snout. The former group contains *A. herklotsi* and *A. longicephalus*, and all the remaining species are included in the latter group.

### Spiral valves

The number of spiral turns in the valvular intestine, which is counted as spiral valve counts, is one of a few reliable meristic characters for the elasmobranch fishes. However, valve counts are not always given in the taxonomic papers on sharks.

We examined 262 specimens in 25 species (Table I). The spiral valve counts ranged from 7 to 22 for the genus, and one species has a narrow range with a maximum of

Table I. - Frequency distribution of spiral valve counts.

Apristurus species	Spiral valve counts															
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
<i>A. aphyodes</i>			8	9	1											
<i>A. atlanticus</i>												1				
<i>A. brunneus</i>									1	9	20	12	4			
<i>A. canutus</i>								2	1	6	2					
<i>A. fedorovi</i>			8	10	4	1										
<i>A. gibbosus</i>												1	1	1		
<i>A. herklotsi</i>				4	5											
<i>A. indicus</i>											1					
<i>A. japonicus</i>												3	4	2		
<i>A. kampae</i>			4	8	4	2										
<i>A. laurussonii</i>											1		1	2		
<i>A. longicephalus</i>							1	1	2	1	1					
<i>A. macrorhynchus</i>										1				2		
<i>A. manis</i>			1	2	7	3										
<i>A. microps</i>			1	7	1											
<i>A. nasutus</i>									4	2	1					
<i>A. parvipinnis</i>													5	5	5	6
<i>A. pinguis</i>	1	3	2	1	1											
<i>A. platyrhynchus</i>										2	2	5	10	5		
<i>A. profundorum</i>				1												
<i>A. riveri</i>		2	10	3												
<i>A. saldanha</i>										1						
<i>A. spongiceps</i>				1												
<i>A. stenseni</i>			1	3	4											
<i>A. verweyi</i>											1					
Total (specimens)	1	5	35	49	27	6	1	3	8	22	30	27	25	16	6	1
Total (species)	1	2	8	11	8	3	1	2	4	7	9	6	6	5	1	1

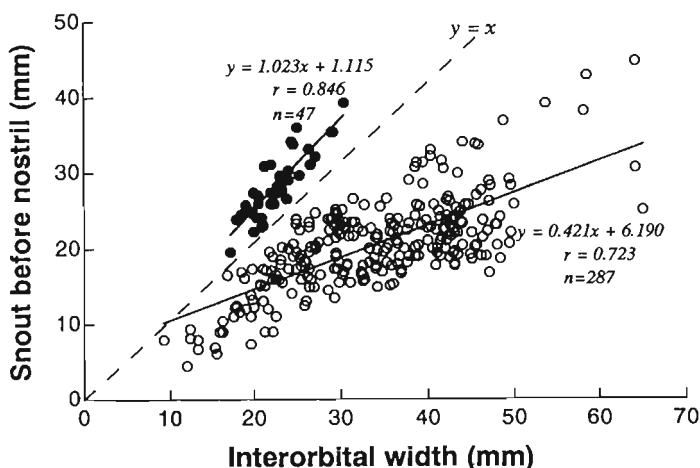


Fig. 2. - Relation of snout length before nostril and interorbital width. ● Long-snouted species; ○ short-snouted species.

5 valves. For example, *A. fedorovi* is characterized by having 9-12 spiral valves, *A. platyrhynchus* by 16-20 valves, and *A. parvipinnis* by 18-22 valves. In addition, as indicated by the number of total species (Table I), there exist two modes (10 and 17) in the number of spiral valve counts, and the groups represented by those modes are composed of different species. The group with lower spiral valve counts is composed of 11 species, such as *Apristurus aphyodes*, *A. fedorovi*, *A. herklotsi*, *A. kampae*, *A. manis*, *A. microps*, *A. pinguis*, *A. profundorum*, *A. riveri*, *A. spongiceps* and *A. stenseni*, and the group with higher counts is represented by 14 species, like *A. atlanticus*, *A. brunneus*, *A. canutus*, *A. gibbosus*, *A. indicus*, *A. japonicus*, *A. laurussonii*, *A. longicephalus*, *A. macrorhynchus*, *A. nasutus*, *A. parvipinnis*, *A. platyrhynchus*, *A. saldanha* and *A. verweyi*.

### Labial furrows

Species of *Apristurus* have distinct upper and lower labial furrows around the corner of the mouth (Fig. 1). The upper labial furrow extends anteriorly from the mouth corner to the nostril, and the lower furrow runs medially along the mouth cleft. Although the lower labial furrows are similar in all the species, the length of the upper labial furrow differs among species.

Some species have long upper labial furrows reaching beyond midway to the nostril, and the lengths of the upper labial furrows are longer than the lower ones (Fig. 1A, C). Others have short upper labial furrows, which are subequal to, or shorter, than the lower furrows (Fig. 1B). The former group includes *Apristurus acanutus*, *A. atlanticus*, *A. brunneus*, *A. canutus*, *A. gibbosus*, *A. herklotsi*, *A. indicus*, *A. internatus*, *A. investigatoris*, *A. japonicus*, *A. laurussonii*, *A. longicephalus*, *A. macrorhynchus*, *A. macrostomus*, *A. micropterygeus*, *A. nasutus*, *A. parvipinnis*, *A. platyrhynchus*, *A. saldanha*, *A. sinensis* and *A. verweyi*. The latter group contains *Apristurus aphyodes*, *A. fedorovi*, *A. kampae*, *A. manis*, *A. microps*, *A. pinguis*, *A. profundorum*, *A. riveri*, *A. spongiceps* and *A. stenseni*.

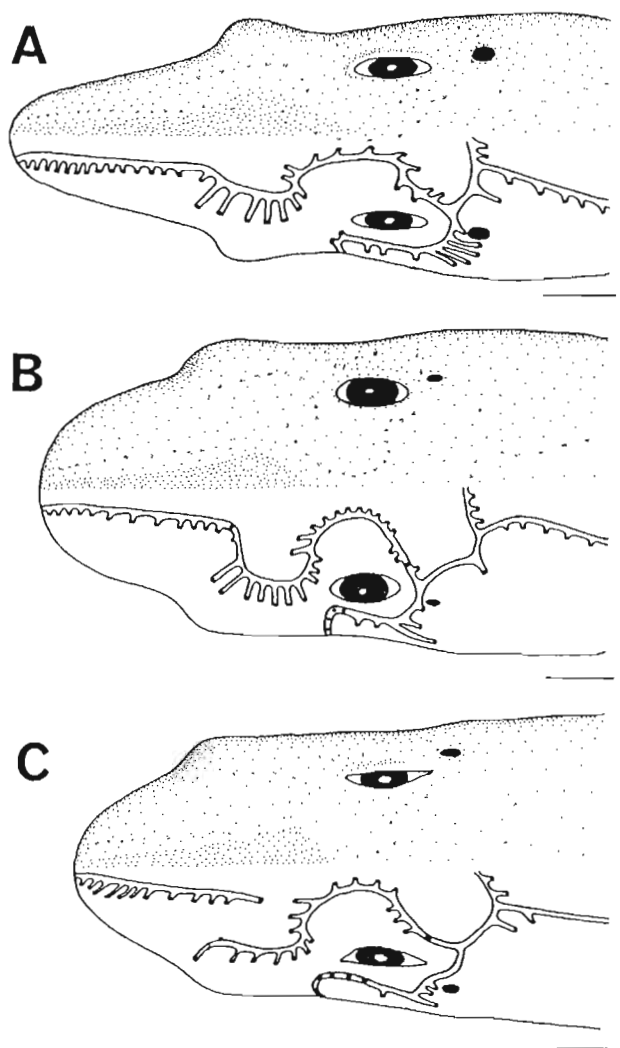


Fig. 3. - Supraorbital sensory canal in *Apristurus herklotsi* (A), *A. fedorovi* (B) and *A. brunneus* (C). Scale bars = 10 mm.

### Cephalic sensory canal

We examined the cephalic sensory canal system in 17 species, and two types were recognized in the patterns of the supraorbital canal. One is a continuous type (Fig. 3A, B), in which the supraorbital canal runs continuously from postorbital region to the snout tip. The other one is a discontinuous type, where the canal from the postorbital region and the canal on the snout are separated above the nasal area (Fig. 3C). The continuous type is seen in *Apristurus aphyodes*, *A. fedorovi*, *A. herklotsi*, *A. kampae*, *A. longicephalus*, *A. manis*, *A. microps* and *A. pinguis*, while the discontinuous type is found in *A.*

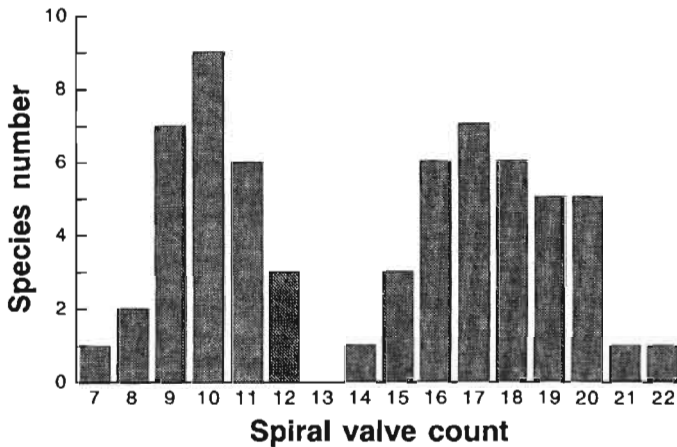


Fig. 4. - Species number histogram by spiral valve counts for short-snouted species.

*brunneus*, *A. canutus*, *A. gibbosus*, *A. japonicus*, *A. laurussonii*, *A. macrorhynchus*, *A. nasutus*, *A. parvipinnis* and *A. platyrhynchus*.

## DISCUSSION

The four characters adopted in this study divide the species of *Apristurus* into three groups. The relationships between length of snout before nostrils and interorbital width (Fig. 2) indicates that *Apristurus herklotsi* and *A. longicephalus* are quite distinct, and are easily separable from the short-snouted species. The short-snouted species include 30 species, but they are further divided into two species groups by the spiral valve counts in the intestine, labial furrows, and shape of the cephalic sensory canal. Spiral valve counts varied widely from 7 to 22, but the figure 4 clearly indicates two groups for the short-snouted species, one with lower spiral valve counts (7-12) and one with higher counts (14-22). The short-snouted species possess either long or short upper labial furrows (Fig. 1). Two types of patterns were recognized in the supraorbital sensory canal (Fig. 3). These three characters could not be obtained for *A. sibogae* because of the poor condition of the type specimen, which is the only existing specimen of the species, but as discussed below, the type specimen has additional characters which are commonly possessed by the short-snouted species. Nakaya (1989) also considered *A. sibogae* to be a species close to *A. platyrhynchus*, and here we follow Nakaya (1989) in the treatment of the species.

Figure 5 shows a combination of labial furrow lengths and spiral valve counts for the short-snouted species, and this result indicates an obvious correlation that the species with higher spiral valve counts (open dots) have long upper labial furrows, and those with lower spiral valve counts (solid dots) have short labial furrows.

Figure 6 is a combination of all the four characters mentioned above. The species with higher spiral valve counts and long upper labial furrows (indicated by open symbols) have consistently discontinuous (marked "D" in Fig. 6) supraorbital canals. On the other hand, the species with lower valve counts and short upper labial furrows (solid symbols)

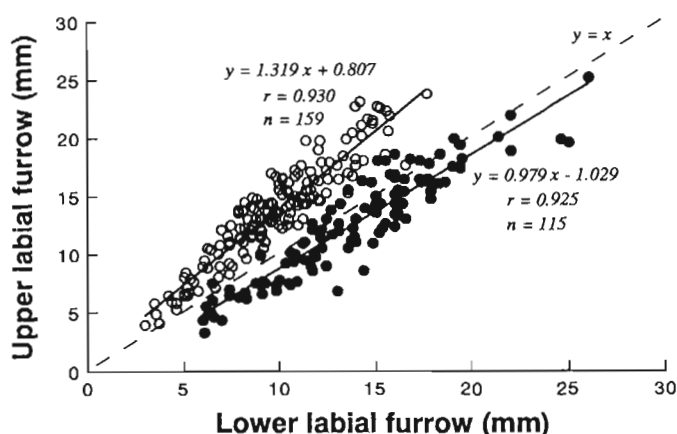


Fig. 5. - Combination of labial furrow lengths and spiral valve counts for short-snouted species. ○: Species with higher (14-22) spiral valve counts; ●: species with lower (7-12) spiral valve counts.

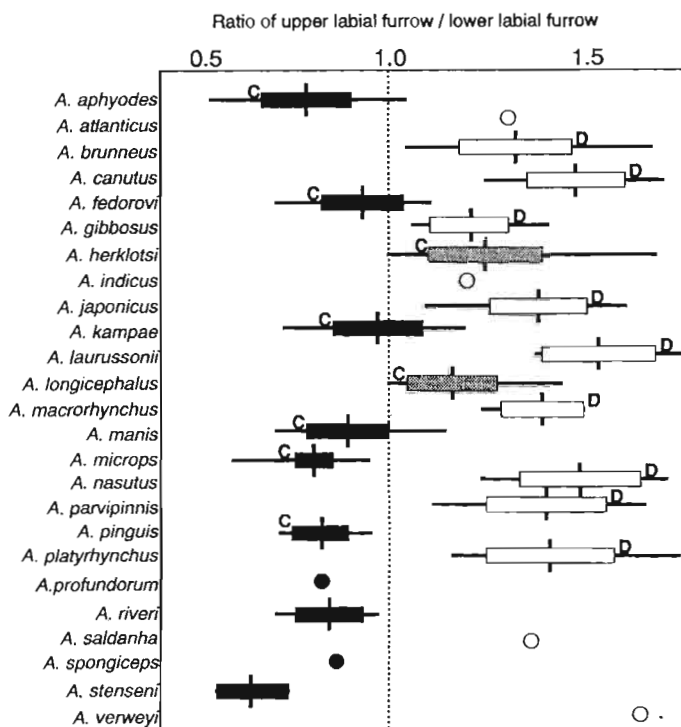


Fig. 6. - Combination of snout lengths, labial furrows, spiral valve counts and supraorbital sensory canal. ○: Species of *brunneus* group; ●: species of *spongeiceps* group; stippled symbols: species of *longicephalus* group; C: species with continuous supraorbital canal; D: species with discontinuous supraorbital canal. Vertical bar: mean; horizontal bar: range; square: standard deviation.



have always continuous ("C") supraorbital canals. For example, *Apristurus fedorovi* which possesses a short upper labial furrow (Fig. 1B) has only 9-12 spiral valves (Table I) and a continuous supraorbital canal (Fig. 3B). *A. brunneus* with a long upper labial furrow (Fig. 1C) has 15-19 spiral valves (Table I) and a discontinuous supraorbital canal (Fig. 3C). Almost perfect coincidence of three independent characters appears to justify the two groups in the short-snouted species.

In addition to the above characters, other characters correspond to the two groups. The species with low spiral valve counts tend to have a stout body, sparse distribution of dermal denticles, small pectoral fin, high anal fin, rounded pectoral and anal fins, and high dorsal fins. On the other hand, the species with high valve counts tend to have a slender body, close distribution of dermal denticles, large and angular pectoral fin, low anal fin, and slender dorsal fins.

Two long-snouted species (stippled symbols in Fig. 6) do not fit for the rule deduced from short-snouted species. They have long upper labial furrows but continuous supraorbital canals. Further, *Apristurus herklotsi* has lower spiral valve counts (10-11), while *A. longicephalus* has higher counts (13-17). However, *A. longicephalus* is quite unique among species of *Apristurus* in having an unusually long duodenum and a considerably short spiral intestine (Nakaya, 1991, fig. 8). The length of the spiral intestine in *A. longicephalus* is almost half of that of the same sized specimens in other *Apristurus* species. Therefore, it seems probable that the spiral valve counts increased secondarily to compensate shortened intestine in *A. longicephalus*. At any rate, *A. herklotsi* and *A. longicephalus* are unique species, separated from the short-snouted species by unusually long snout.

Based on the discussion above, we recognize three morphological groups in the genus *Apristurus*, i.e., long-snouted species group (*longicephalus* group), and two short-snouted groups (*brunneus* group with higher spiral valve counts, long upper labial furrows and discontinuous supraorbital sensory canal; *spongiceps* group with lower spiral counts, short upper labial furrows and continuous supraorbital canal). Compagno (1988) used the above three group names, but our *brunneus* and *spongiceps* groups are completely different from those of Compagno (1988), though our *longicephalus* group is almost equal to his *longicephalus* group.

As shown above, the spiral valve counts range from 7 to 22 in this genus, but the range of variation (16) seems to be too large for one genus. Variation in one genus is usually less than 6 in the Carcharhiniformes (Compagno, 1988). The largest variation (9) is seen in a scyliorhinid genus, *Galeus*. The spiral valve counts in the triakid genus *Mustelus*, which is one of the largest genera with at least 15 species, range only 6. In addition, the persistent coincidence of independent characters within *brunneus* group and also within *spongiceps* group may suggest certain genealogical implication of such groups. Although these facts may imply some taxonomic changes are required within the genus, we refrain from changing taxonomic status of the genus *Apristurus*, or giving taxonomic ranks to these groups, until their phylogenetic relationships are fully clarified.

#### Definition of the species groups

##### *longicephalus* group

Snout narrow and long; length before anterior nostrils longer than 6.4% TL, distinctly greater than interorbital width (1.02-1.46 times the latter). Species: *herklotsi* and *longicephalus*;

***brunneus* group**

Snout wide and short; length before anterior nostrils usually less than 6.0% TL, much less than interorbital width (0.36-0.94 times the latter). Spiral valves 13-22; upper labial furrows apparently longer than lower furrows; supraorbital sensory canal discontinuous. Species: *acanutus*, *atlanticus*, *brunneus*, *canutus*, *gibbosus*, *indicus*, *internatus*, *investigatoris*, *japonicus*, *laurussonii*, *macrorhynchus*, *macrostomus*, *micropterygeus*, *nasutus*, *parvipinnis*, *platyrhynchus*, *saldanha*, *sibogae*, *sinensis* and *verweyi*;

***spongiceps* group**

Snout wide and short; length before anterior nostrils usually less than 6.0% TL, much less than interorbital width (0.36-0.94 times the latter). Spiral valves in the intestine 7-12; upper labial furrows subequal to, or shorter than, lower furrows; supraorbital sensory canal continuous. Species: *aphyodes*, *fedorovi*, *kampae*, *manis*, *microps*, *pinguis*, *profundorum*, *riveri*, *spongiceps* and *stenseni*.

**List of specimens examined**

*Apristurus abbreviatus* Deng, Xiong & Zhan, 1985: Holotype. - ECSFI (East China Sea Fisheries Institute, Shanghai) E-1547, 430 mm TL, male, East China Sea; Paratypes. - ECSFI E-1000, ECSFI E-1001, ECSFI E-1417, ECSFI 1548, ECSFI E-1597, 311-407 mm TL, 2 males and 3 females, East China Sea.

*Apristurus acanutus* Chu, Meng & Li, 1985: Holotype. - SCSFRI (South China Sea Fisheries Research Institute, Guangzhou) D-172, 522 mm TL, female, South China Sea; Paratype. - SFC (Shanghai Fisheries College) D161, 520 mm TL, female, South China Sea.

*Apristurus aphyodes* Nakaya & Stehmann, 1998: Holotype. - ISH 71-1981, 538 mm TL, male, Lousy Bank, Eastern North Atlantic; Paratypes. - ISH 807-1974, ISH 807-1974, ISH 24-1981, ISH 36-1981, ISH 49-1981, ISH 84-1981, ISH 124-1981, ISH 184-1983, ISH 187-1983, HUMZ 152330, USNM 347837, ZIN N51551, BMNH 1998.1.22.1, 209.2-540.0 mm TL, 8 males and 9 females, eastern North Atlantic.

*Apristurus atlanticus* (Koefoed, 1927): Holotype. - ZMUB 3203, 247 mm TL, male, eastern Atlantic near Canary Islands.

*Apristurus breviceaudatus* Chu, Meng & Li, 1985: Holotype. - SCSFRI D-1125, 397 mm TL, male, South China Sea; Paratypes. - SFC D-32, SFC D-1126, 412-419 mm TL, 2 males, South China Sea.

*Apristurus brunneus* (Gilbert, 1891): Holotype. - USNM 51708, 478 mm TL, male, off La Jolla, California; 69 other specimens, CAS 13425, CAS 15323, CAS 15323-3, CAS 15323-4, CAS 15325-1, CAS 15325-2, CAS 40240, CAS 40241, CAS 40242, CAS 40243 (2 spms), CAS 40252 (4 spms), CAS 56248 (4 spms), CAS 58920, FSFL 568-1, FSFL 568-2, FSFL 568-3, FSFL 568-4, FSFL 568-5, FSFL 568-6, HUMZ 105584, HUMZ 105585, HUMZ 105586, HUMZ 105587, HUMZ 105588, HUMZ 105589, HUMZ 105590, HUMZ 105592, HUMZ 105593, HUMZ 105594, HUMZ 105595, HUMZ 105598, HUMZ 105599, HUMZ 105600, HUMZ 105610, HUMZ 105611, HUMZ 110327, HUMZ 30696, HUMZ 30697, HUMZ 30710, LACM W53-4, LACM W53-4(5), LACM 30378-1, LACM 30797-2 (2 spms), LACM 30803-1, LACM 30806-1 (2 spms), LACM 30808-1, LACM 31881-1, LACM 38257-1, LACM 39105-0, LACM 53-4, SIO 81-3, SIO 83-97, USNM 188033 (2 spms), 130-648 mm TL, 43 males and 26 females, Californian waters.

*Apristurus canutus* Springer & Heemstra, 1979: Holotype. - USNM 206176, female, 451 mm TL, Lesser Antilles; Paratypes. - USNM 206180, 3 males and 1 female, 318-433 mm TL, Lesser Antilles; 9 other spms, USNM 221293, 221294 (2 spms), USNM 221295, USNM 221297, USNM 221299, USNM 221454, ZMB 31556, 208-436 mm TL, 4 males and 5 females, Caribbean Sea.

*Apristurus fedorovi* Dolganov, 1985: 28 spms, HUMZ 40074, HUMZ 69163, HUMZ 72690, HUMZ 72716, HUMZ 72871, HUMZ 73037, HUMZ 74628, HUMZ 78038, HUMZ 78052, HUMZ 78064, HUMZ 78071, HUMZ 78100, HUMZ 78101, HUMZ 78105, HUMZ 78140, HUMZ 78174, HUMZ 78195, HUMZ 78196, HUMZ 78260, HUMZ 78276, HUMZ 78281, HUMZ 78316, HUMZ 78318, HUMZ 78333, HUMZ 78334, HUMZ 78374, HUMZ 78454, HUMZ 85087, 320-683 mm TL, 15 males and 13 females, northern Japanese waters.

*Apristurus gibbosus* Meng, Chu & Li, 1985: Holotype. - SCSFRI D1121, 425 mm TL, female, South China Sea; Paratypes. - SFC D-84, SFC D-1133, 375-390 mm TL, 2 females, South China Sea; 10 other spms, SFU D-0094, SFU E-1800097, SFU D-0300, SFU D-2268, SFU D-0339, SFU E-0174, SFU D-0700, HUMZ 145164, HUMZ 145166, HUMZ 145171, 309-542 mm TL, 7 males and 3 females, East China Sea.

*Apristurus herklotzi* (Fowler, 1933): Holotype. - USNM 93134, 326 mm TL, female, Cagayan Island, Jolo Sea, Philippines; 22 other spms, BSKU 23109, BSKU 23110, BSKU 26647, BSKU 27598, BSKU 27882, ZIUT 10142, ZIUT 10143, ZIUT 10144, 311-485 mm TL, 3 males and 5 females, Japanese waters.

*Apristurus indicus* (Brauer, 1906): Lectotype - ZMB 22424, 364 mm TL, female, western Indian Ocean off Somalia; Paralectotype - ZMB 17411, female, western Indian Ocean off Somalia.

*Apristurus internatus* Deng, Xiong & Zhan, 1988: Holotype. - ECSFI SH80D-0316, 419 mm TL, female, East China Sea; Paratype. - ECSFI E-1226, 403 mm TL, male, East China Sea.

*Apristurus investigatoris* (Misra, 1959): Holotype. - ZSI Fi 627/2, 243 mm TL, female, Andaman Sea.

*Apristurus japonicus* Nakaya, 1975: Holotype. - HUMZ 40082, 697 mm TL, male, Choshi, Japan; Paratypes. - HUMZ 39961, HUMZ 40075, HUMZ 40076, HUMZ 40077, HUMZ 40078, HUMZ 40079, HUMZ 40080, HUMZ 40081, 626-711 mm TL, 7 males and 1 female, Choshi, Japan; 8 other spms (no catalog number given at HUMZ), 457-705 mm TL, 6 males and 2 females, Choshi, Japan.

*Apristurus kampa* Taylor, 1972: Holotype. - SIO 70-248-5, 348 mm TL, female, Gulf of California; 31 other spms, CAS 38287, CAS 57935, HUMZ 105601, HUMZ 105603, HUMZ 105604, HUMZ 105608, HUMZ 110317, HUMZ 110318, HUMZ 110319, HUMZ 110320, HUMZ 110321, HUMZ 110322, HUMZ 110323, HUMZ 110324, HUMZ 110325, HUMZ 110326, HUMZ 110330, HUMZ 110331, HUMZ 110332, HUMZ 110333, HUMZ 110334, HUMZ 110335, HUMZ 110336, HUMZ 110337, LACM 37499-1, SIO 70-299, SIO 71-190, SIO 88-100, SIO 88-98, SIO 99-99, 198-584 mm TL, 16 males and 15 females, Californian waters and Galapagos Islands.

*Apristurus laurussonii* (Saemundsson, 1922): Holotype. - NHMR (no catalog number given), 663 mm TL, female, Icelandic waters; 4 other spms, ISH 51-1965, ISH 23-1981 (2 spms), ISH 109-1981, 592-719 mm TL, eastern North Atlantic.

*Apristurus longianalis* Chu, Meng & Li, 1986: Holotype. - SCSFI S-6530, 366 mm TL, female, South China Sea; Paratype. - SFC D-571, 359 mm TL, female, South China Sea.

*Apristurus longicaudatus* Li, Meng & Chu, 1986: Holotype. - SCSFI D-811, 324 mm TL, male, South China Sea; Paratype. - SFC 564, 330 mm TL, male, South China Sea.

*Apristurus longicephalus* Nakaya, 1975: Holotype. - HUMZ 42399, 367 mm TL, male, Tosa Bay, Japan; 25 other spms, BSKU 26867, BSKU 26512, BSKU 28166, BSKU 26648, BSKU 26455, BSKU 22338, BSKU 28096, BSKU 33999, BSKU 28097, BSKU 26651, BSKU 33520, BSKU 33519, BSKU 26868, BSKU 34000, BSKU 27596, BSKU 23012, BSKU 33518, BSKU 26649, BSKU 26650, 6 spms of P.P. Shirshov Inst. (uncatalogued), 253-595 mm TL, 22 males and 3 females, southern Japanese waters and southwest Indian Ocean.

*Apristurus macrorhynchus* (Tanaka, 1909): Holotype. - ZUMT 2153, 444 mm TL, male, Misaki, Japan; 5 other spms, BSKU 26574, BSKU 32566, ZIUT 3467, HUMZ 168185, 142-674 mm TL, 2 males and 3 females, southern Japanese waters.

*Apristurus macrostomus* Chu, Meng & Li, 1985: Holotype. - SCSFI D-807, 389 mm TL, male, South China Sea.

*Apristurus maderensis* Cadenat & Maul, 1966: Holotype. - MMF (Museu Municipal do Funchal, Madeira, Portugal) 18750, 665 mm TL, female, Madeira.

*Apristurus manis* (Springer, 1979): Holotype. - MCZ 38299, 390 mm TL, female, waters off Massachusetts; Paratypes. - MCZ 37416 (2 spms), MCZ 37512, MCZ 37535, 227-255 mm TL, 3 males and 1 female, waters off Massachusetts; 11 other spms, ARC 8601097, ARC 8602997, ISH 154-1974a, ISH 154-1974b, ISH 3412-1979, ISH 3449-1979, ISH 3712-1979, ISH 3713-1979(2 spms), MCZ 37407, 1 spm of P.P. Shirshov Inst. (uncatalogued), 183.2-852.1 mm TL, 4 males and 7 females, North Atlantic.

*Apristurus microps* (Gilchrist, 1922): MCZ 58434, ISH 943-1973, ISH 944-1973, ISH 698-1974 (2 spms), ISH 697-1974, ISH 49-1981, ISH 945-1973, ISH 195-1967 (2 spms), 259.3-730 mm TL, 4 males and 7 females, North Atlantic and South African waters.

*Apristurus micropterygeus* Meng, Chu & Li, 1986: Holotype. - SCSFI E-1128, 381 mm TL, male, South China Sea.

*Apristurus nasutus* Buen, 1959: 13 spms, MNHNC-P 6502, MNHNC-P 6503 (2 spms), MNHNC-P 6504, MNHNC-P 6505, MNHNC-P 6506 (2 spms), MNHNC-P 6507, MNHNC-P 6508, MNHNC-P 6509, MNHNC-P 6510, MNHNC-P 6512, USNM 221516, 171-557 mm TL, 6 males and 7 females, Chilean waters.

*Apristurus parvipinnis* Springer & Heemstra, 1979: Holotype. - USNM 206178, 472 mm TL, male, Gulf of Mexico; Paratypes. - USNM 206179, USNM 200969, 403-466 mm TL, 2 females, Gulf of Mexico; 25 other spms, MCZ 40249, UF 27946, 39943 (2 spms), 45231 (2 spms), USNM 165557, USNM 201906 (2 spms), USNM 221451, USNM 221487, USNM 221488, USNM 221489, USNM 221490, USNM 221496, USNM 221500, USNM 221502, USNM 221508 (2 spms), USNM 221537, USNM 221639, USNM 221640 (3 spms), ZMB 31555, 258-520 mm TL, 12 males and 13 females, Caribbean and Gulf of Mexico.

*Apristurus pinguis* Deng, Xiong & Zhan, 1983: Holotype. - ECSFI SH80D-312, 558 mm TL, male, East China Sea; 8 other spms, HUMZ 145143, HUMZ 145144, HUMZ 145145, HUMZ 145146, HUMZ 145147, HUMZ 145148, HUMZ 145149, HUMZ 145150, 278-548 mm TL, 5 males and 3 females, East China Sea.

*Apristurus platyrhynchus* (Tanaka, 1909): 31 spms, BSKU 22337, BSKU 22788, BSKU 26866, BSKU 27062, BSKU 27063, BSKU 27065, BSKU 27595 (2 spms), BSKU 27597, BSKU 27964, BSKU 33521, BSKU 33522, BSKU 33523, BSKU 33524, BSKU

33525, BSKU 33972, BSKU 43664, HUMZ 103699, HUMZ 103700, HUMZ 105984, HUMZ 105985, TMFE 21, TMFE 22, TMFE 23, TMFE 40, TMFE 286, TMFE 287, TMFE 520, TMFE 591, TMFE 592, ZIUT 3424, 280-739 mm TL, 13 males and 18 females, Japanese waters.

*Apristurus profundorum* (Goode & Bean, 1895): Holotype. - USNM 35646, 510 mm TL, male, off Delaware Bay.

*Apristurus riveri* Bigelow & Schroeder, 1944: Holotype. - MCZ 36092, 413 mm TL, female, waters off north of Cuba; 16 other spms, USNM 199395, USNM 199396, USNM 201760 (2 spms), USNM 221526 (2 spms), USNM 221528, USNM 221530, USNM 221531, USNM 221533 (2 spms), USNM 221535, USNM 221536 (2 spms), USNM 221760 (2 spms), 298-470 mm TL, 7 males and 9 females, Caribbean and Gulf of Mexico.

*Apristurus saldanha* (Barnard, 1925): 2 spms, BMNH 1935.5.2.56-58, 451-408 mm TL, 1 male and 1 female, off the south-western Cape, South Africa.

*Apristurus sibogae* (Weber, 1913): Holotype. - ZMA 111.076, 228 mm TL, female, Makassar Strait, Indonesia.

*Apristurus sinensis* Chu & Hu, 1981: Holotype. - SCSFI 99, 426 mm TL, male, South China Sea.

*Apristurus spongiceps* (Gilbert, 1905): Holotype. - USNM 51590, 514 mm TL, female, Hawaii.

*Apristurus stenseni* (Springer, 1979): Holotype. - ZMUC-P 6146, 185 mm TL, male, eastern Pacific in Gulf of Panama; Paratypes. - ZMUC-P 6147, ZMUC-P 6148, ZMUC-P 6159, ZMUC-P 6162, ZMUC-P 6164, ZMUC-P 6166, ZMUC-P 6173, ZMUC-P 6182, ZMUC-P 6189, HUMZ 138785, HUMZ 138786, 118-228 mm TL, 5 males, 5 females and 1 spm of unknown sex, eastern Pacific in Gulf of Panama.

*Apristurus verweyi* (Fowler, 1933): Holotype. - USNM 93135, 303 mm TL, male, Sibuko Bay, Borneo.

*Apristurus xenolepis* Meng, Chu & Li, 1985: Holotype. - SCSFRI D-42, 415 mm TL, female, South China Sea.

**Acknowledgements.** - We wish to express our sincere gratitude to the following persons. Dr. K. Amaoka gave advice and encouragements during the research. Drs. M.-L. Bauchot, E. Bertelsen, M.J. Biscoito, G.H. Burgess, I. Brykjedal, R. Daley, S.M. Deng, J.A.F. Garrick, A. Graham, K.E. Hartel, G. Langhelle, P. Last, S. Li, G.E. Maul, R. Meléndez C., Q.W. Meng, N.R. Merrett, R. Muños-Chapuli, J. Nielsen, H.-J. Paepke, N.V. Parin, C. Paulin, G. Pequeño, A. Petersen, P. Pethon, C.D. Roberts, B. Séret, K. Suluk, V.G. Springer, M. Stehmann, J.D. Stevens, A. Stewart, S. Tanaka, T. Taniuchi, A. Wheeler, H.L. Wu, G.Q. Xiong, and H.G. Zhan gave us permission to examine the types and other specimens in their institution and helped us in many ways.

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## GEOGRAPHICAL VARIATION IN AGE AND GROWTH OF *SQUALUS MITSUKURII* (ELASMOBRANCHII: SQUALIDAE) IN THE NORTH PACIFIC

by

Toru TANIUCHI (1) & Hiroyuki TACHIKAWA (2)

**ABSTRACT.** - Estimates of age and growth of three North Pacific populations of *Squalus mitsukurii* were compared, i.e., that of Choshi (appr. 36°N, 141°E, n = 38 females, 85 males), that situated near Ogasawara Islands (appr. 27°N, 142°E, n = 130 females, 54 males), and that in the southeast of the Hancock Seamount (appr. 30°N, 180°E, n = 36 females, 28 males). Annuli in the second dorsal spine were used to provide age estimates. Annuli were assumed to be formed once a year according to the former researches conducted for several species of *Squalus*. Von Bertalanffy growth equations were calculated based on a mean total length for each age. Asymptotic lengths for females were larger than for males in each locality, and growth coefficients are smaller for males than for females. These two growth parameters showed geographical variations. The asymptotic length, in both sexes, tended to be largest for the Choshi population and smallest for that of the Hancock Seamount. On the contrary, the growth coefficient of males tended to be highest for the Hancock Seamount population and lowest for that of Choshi; for the females this coefficient was highest for the Hancock Seamount population, and lowest for that of Ogasawara Islands. Age at sexual maturity for the females was estimated to be about age 19-20 for the Choshi population, age 15-17 for that of Ogasawara, and 14-16 for that of SE Hancock Seamount; the maturity for males were age 10-11 for the Choshi population, age 9-10 for that of Ogasawara, and 6-7 for that of the Hancock Seamount.

**RÉSUMÉ.** - Variations géographiques de l'âge et de la croissance de *Squalus mitsukurii* (Elasmobranchii: Squalidae) dans le Pacifique Nord.

Les estimations de l'âge et de la croissance de trois populations de *Squalus mitsukurii* du Pacifique Nord ont été comparées; celle de Choshi (environ 36°N, 141°E, n = 38 femelles, 85 mâles), celle qui est située à proximité des Îles Ogasawara (environ 27°N, 142°E, n = 130 femelles, 54 mâles), et celle du versant sud-est du mont sous-marin Hancock (environ 30°N, 180°E, n = 36 femelles, 28 mâles). Les annuli de la seconde épine dorsale ont été utilisés pour estimer l'âge. Ces annuli se forment apparemment un fois par an, selon les résultats des recherches antérieures menées sur diverses espèces de *Squalus*. Les équations de von Bertalanffy ont été établies à partir des tailles moyennes de chaque classe d'âge. Les tailles asymptotiques des femelles sont généralement plus grandes que celles des mâles, et les taux de croissance sont plus faibles pour les mâles que pour les femelles. Ces deux paramètres de croissance variaient en fonction de l'origine géographique de la population étudiée. Pour les deux sexes, les tailles asymptotiques sont généralement plus grandes dans la population de Choshi et plus petites pour celle du mont sous-marin Hancock. À l'opposé, les taux de croissance des mâles tendent à être plus élevés dans la population du mont sous-marin Hancock et plus faibles dans celle de Choshi; pour les femelles, ces taux sont plus élevés dans la population du mont sous-marin Hancock et plus faibles dans celle des Îles Ogasawara. Chez les femelles, la maturité sexuelle est atteinte pour la classe d'âge 19-20 dans la population de Choshi, la classe d'âge 15-17 pour celle d'Ogasawara, et la classe

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d'âge 14-16 pour celle du mont sous-marin Hancock; pour les mâles, les classes d'âges respectives sont 10-11 (Choshi), 9-10 (Ogasawara) et 6-7 (Mont sous-marin Hancock).

Key-words. - Squalidae, *Squalus mitsukurii*, North Pacific, Age, Growth, Geographical variation.

In a number of local areas, some elasmobranch stocks have been reported to be reduced to a level of extinction by overfishing. However, stock assessments have rarely been attempted for elasmobranchs, partly because they usually are not targeted by commercial fisheries and partly because their basic biological information is poorly known. In stock assessment, age and growth studies are necessary for yield per recruit model and catch-at-age analysis. However, age determination of elasmobranchs was regarded to be difficult in the past because there were no appropriate age characters such as scales and otoliths as found in teleostean fishes. Recently great strides in the development of age techniques for elasmobranchs, particularly for sharks, have been made and a number of age and growth studies have been published in recent years (see Cailliet, 1990, for review). There are two primary hard parts used in elasmobranchs, i.e., the vertebrae and dorsal spines. For dogfish sharks of the family Squalidae, vertebral centra generally have been found unsuitable for age determination because of their calcification pattern, although a recent paper reported success of age determination of *Squalus blainvillei* using this approach (Cannizaro *et al.*, 1995). The preferred approach has therefore used dorsal spine.

At least five species of the genus *Squalus* occurred around Japan. Among these, *S. mitsukurii* is most widely distributed from Hokkaido through Honshu to Kyushu and abundant when compared with other *Squalus* species except *S. acanthias*, which is easily distinguished from all other congeners in diagnostic external characters and distribution. In taxonomic review of the Japanese dogfish (Chen *et al.*, 1979), the occurrence of this species at NE Hancock Seamount in the North Pacific was confirmed. However, we strongly feel that a new world-wide review of the genus *Squalus* is needed because of several unidentified species found even around Japan since that initial review. Munoz-Chapuli and Ramos (1989) have also questioned the validity of *S. mitsukurii*. For the present manuscript we follow the classification of Chen *et al.* (1979).

Several biological studies on *S. mitsukurii* have been published over the years, e.g., Chen (1977) for age, growth, reproduction and food habits, Taniuchi *et al.* (1993) for some aspects of reproduction and by Litvinov (1990) and Wilson and Seki (1994) for growth, reproduction and population characteristics. Taniuchi *et al.* (1993) reported that there are distinct geographical variations in size at maturity and fecundity among four regions in the North Pacific, using the same materials collected from the same regions in this study. The purpose of this study is to describe age and growth for three populations of *S. mitsukurii* and to compare the geographical changes in growth parameters between them.

## MATERIALS AND METHODS

The locality and the number of specimens of *S. mitsukurii* examined in this study are shown in figure 1. Depth ranges were 100-300 m in Choshi, 75-700 m in Ogasawara, and 263-290 m on the SE Hancock Seamount. Sampling gear was mainly trawl in Choshi and SE Hancock Seamount, and pole and line in Ogasawara. The speci-



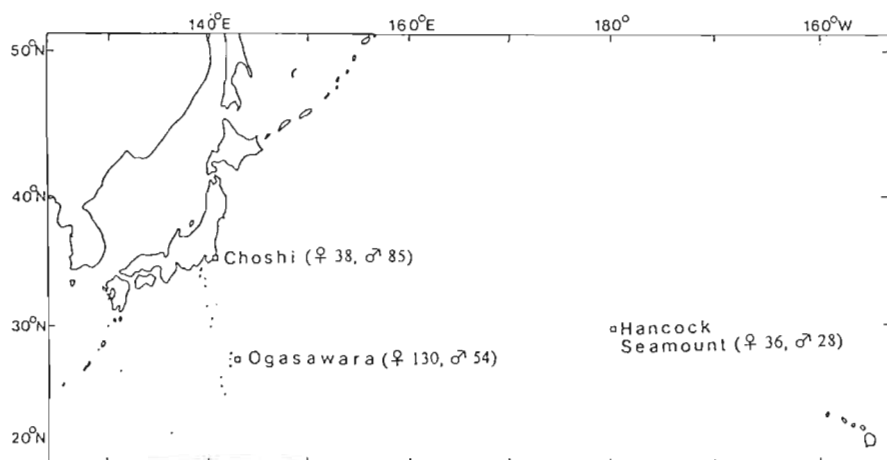


Fig. 1. - Sampling locality of *Squalus mitsukurii* in this study. The numerals in brackets indicate the number of specimens examined for age and growth study.

mens were collected in different periods, i.e., March 1973 for those of the Hancock Seamount, from June 1982 to December, 1983 for those of Choshi, and March 1982 to January 1985 for those of Ogasawara Isl.

When yearly differences in growth occurred within the same locality, direct comparison for growth between localities was not possible. For this study, we assumed that there was no remarkable change in growth between the years 1973 and 1985.

The second dorsal spine was used to obtain age estimates according to Holden and Meadows (1962), Ketchen (1975), and Chen (1977). The number of dark rings observed on the surface of the spine was counted for each specimen. At the time of reading, a ring which seemed to be false was subjectively rejected. However, interpretation of whether two closely spaced bands represented true annuli was dependent on the reader. Spines with two of three identical readings were adopted. No firm criteria were established for readings of the band pattern as described by Ketchen (1975), although Holden and Meadows (1962) established a number of criteria for their readings. To validate formation of annulus, we followed the method conducted by Holden and Meadows (1962) for *Squalus acanthias*, who classified the spine base into three types, dark, light, and wide light (Fig. 2). Each type was expressed as percent occurrence by month.

Data were fitted to von Bertalanffy growth equation, which commonly has been used in modeling for fish growth. Growth parameters were obtained from mean length at each age.

## RESULTS

For the Ogasawara material, where specimens were sampled over several months, no well-defined peak was detected for any basal type probably because of bias of sampling months. Therefore, we failed to conclude dark bands as annuli. Instead, we followed the result of validation of annulus conducted by Chen (1977) for *Squalus japonicus*, which belongs to the same species group as *S. mitsukurii* (Chen *et al.*, 1979). Chen (1977)

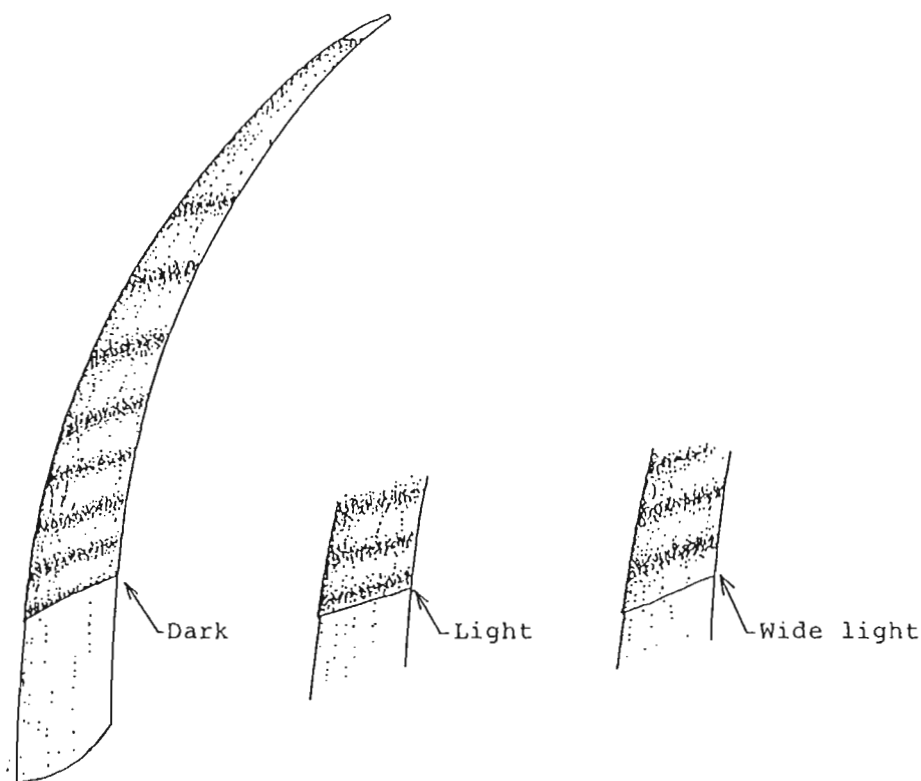


Fig. 2. - Three types of base of second dorsal spine in *Squalus mitsukurii*.

demonstrated that three types showed their peak occurrence in specified months. Also, McFarlane and Beamish (1987) succeeded in the validation of age in *S. acanthias*. Therefore, we tentatively considered dark bands as annuli in this study.

Percentage of agreement of readings was 67.4% in Choshi, 52.1% in Ogasawara, and 60.8% in Hancock Seamount. Low percent agreement in Ogasawara relative to Choshi and Hancock specimens may be attributed to the large size of animals, as was the case in older fishes in general. The real age was estimated as the number of dark rings less one because one dark band already formed before birth. This is the case in most viviparous sharks.

The von Bertalanffy growth equations by sex were obtained for the three localities as described below, where  $t$  = age in years and  $LT$  = total length in mm at age  $t$ :

Choshi population

$$\text{females: } LT = 1628(1 - e^{-0.039(t + 5.21)})$$

$$\text{males: } LT = 1093(1 - e^{-0.066(t + 5.03)})$$

Ogasawara Islands population

$$\text{females: } LT = 1112(1 - e^{-0.051(t + 5.12)})$$

$$\text{males: } LT = 880(1 - e^{-0.060(t + 5.57)})$$

Hancock Seamount population

$$\text{females: } LT = 831(1 - e^{-0.103(t + 2.94)})$$

$$\text{males: } LT = 645(1 - e^{-0.252(t + 0.430)})$$

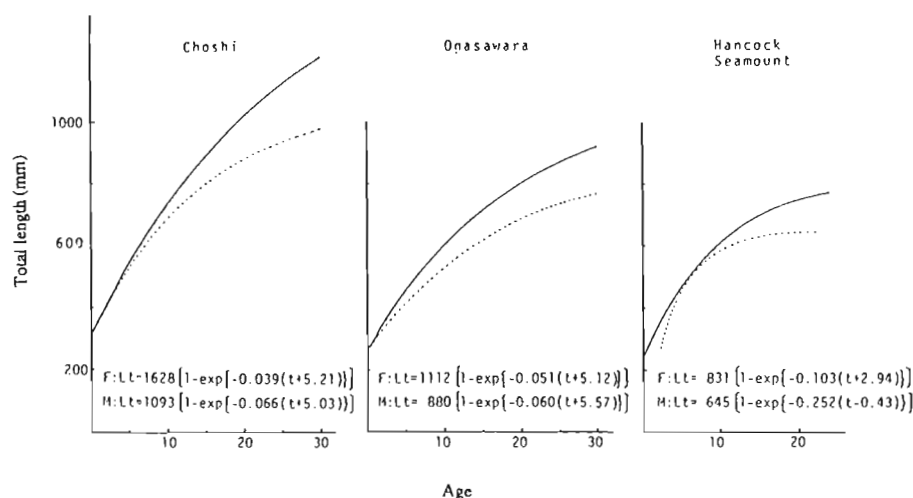


Fig. 3. - Von Bertalanffy growth curves of *Squalus mitsukurii* for three localities by sex. Solid line = female; dotted line = male.

The equations by sex are depicted in figure 3 for the three localities. There are differences in growth parameters between sexes. Males tended to have higher growth coefficients than females in all localities. On the other hand, females seemed to possess larger asymptotic lengths than males. The difference in the asymptotic length between sexes was largest in Choshi.

We compared parameters related with age and growth between localities. As a result, we found geographical variation in two growth parameters. The asymptotic size in total length tended to be largest in Choshi whereas the smallest at SE Hancock Seamount for both sexes. Maximum observed lengths were 125 cm for females and 92 cm for males in Choshi, 96 cm for females and 77 cm for males in Ogasawara, and 73 cm for females and 63 cm for males at SE Hancock Seamount. On the contrary, the growth coefficient appeared to be highest at SE Hancock Seamount whereas the lowest in Choshi for males, but it was highest at SE Hancock Seamount whereas the lowest in Ogasawara for females. The maximum age observed was 21 for females and 20 for males in Choshi, 27 for females and 21 for males in Ogasawara, and 17 for females and 12 on the Hancock Seamount.

Table I. - Size and age at maturity for each locality in *Squalus mitsukurii*. Size range of maturity was cited from Taniuchi *et al.* (1993). n = number of specimens.

	Females			Males		
	Size range (mm)	Age	n	Size range (mm)	Age	n
Choshi	1,000-1,039	19-20	38	680-719	10-11	85
Ogasawara	720-759	15-17	130	520-559	9-10	54
SE Hancock Seamount	680-719	14-16	36	480-519	6-7	28

## DISCUSSION

Geographical variations have been frequently reported not only in morphological characteristics but also in life-history traits in sharks. In the case of the genus *Squalus*, particularly in *S. acanthias*, several subspecies were proposed based upon pectoral fin length (Lindberg and Lezega, 1957). However, subspecies status of *S. acanthias* is now invalid for the reason that the difference in the pectoral fin length is caused by intraspecific variation (Garrick, 1960; Forrester, 1972). Several stocks of *S. acanthias*, which suggest the existence of some geographical variations in morphology, life history, and genetics, are believed to occupy the British Columbia waters (Ketchen, 1986). Geographical variations in reproductive parameters were also reported in *S. acanthias* by Ketchen (1986), *S. japonicus* by Chen *et al.* (1981) and *S. mitsukurii* by Taniuchi *et al.* (1993). Taniuchi *et al.* (1993) showed that a common linear relationship between total length and number of progeny in *S. mitsukurii* among the three localities and they concluded that this shark has a common reproductive strategy regarding fecundity within the species, but that each population shows a characteristic fecundity schedule probably influenced by local abiotic and/or biotic environments. The similar patterns and strategy were observed in *S. japonicus* (Chen *et al.*, 1981; Taniuchi, 1991). Litvinov (1990) suggested the existence of reproductively isolated subpopulations around southeastern Pacific Ocean.

Age and growth estimates of *S. mitsukurii* have been previously reported from Choshi (Chen, 1977), southeastern Pacific (Litvinov, 1990), and central North Pacific (Wilson and Seki, 1994). These studies also failed to validate annulus formation primarily because of sampling bias and / or the small number of specimens examined. Comparison of growth parameters in Choshi between Chen's and our studies revealed that both the growth coefficient and asymptotic length showed a slightly higher values in the present study than Chen's (1977). The difference was attributable to the reading method rather than to difference in the duration of collecting material because there were only 7-8 years in time difference between the two studies.

Litvinov (1990) stated there was definite sexual dimorphism in the relationship between the number of bands or rings and body length; i.e., more variability in the number of marks in males and less in females. The maximum age for males was 14 years and 16 years for females. Since he did not present any growth formula, a direct comparison of growth cannot be undertaken. Wilson and Seki (1994) presented von Bertalanffy growth equations at the Hancock Seamount based on second dorsal spines from 102 males and 105 females. Asymptotic length was 107 cm for females (much larger than 83 cm in our study) and 66 cm for males (equivalent to 65 cm in our study). The growth coefficient was 0.041 (0.103 in our study) for females and 0.155 (0.252 in our study) for males. Growth parameters obtained by Wilson and Seki (1994) produced longer observed life span (27 years) than in our study (17 years), even though we took into consideration that our largest specimen was 72.5 cm (80 cm in their study). The difference in growth parameters might be attributable to the reading pattern. Cailliet *et al.* (1990) pointed out that the population and/or environmental effects cannot be confidently accounted for the suggested difference in growth curves among populations. It is important to establish firm criteria in reading bands.

Our study enables us to make a direct comparison of age and growth between localities because we read bands based on our own criteria. We found geographical variation in growth parameters among the three localities, Choshi, Ogasawara and SE Hancock

Seamount. The separation of the populations was suggested from Taniuchi *et al.* (1993) from the viewpoint of a reproductive strategy.

The combination of reproduction and growth studies revealed that age at maturity was also different among localities. Taniuchi *et al.* (1993) reported that sizes at maturity were the 960-999 mm class interval for females and 680-719 mm for males in Choshi, equivalent to ages 19-20 and 10-11 respectively when we converted lengths to ages according to growth equations. Sizes at maturity were reported to be 720-759 mm class interval for females and 520-559 mm for males in Ogasawara, equivalent to ages 15-17 and 9-10 respectively. Sizes at maturity was estimated to be 680-719 mm for females and 520-559 mm for males in SE Hancock Seamount, equivalent to ages 14-16 and 6-7 respectively, although the number of mature specimens was very few. In conclusion, ages at maturity in Choshi were older than in the remaining two localities for both sexes among the three by 3-5 years. The reason why Choshi specimens reached at maturity at older ages may be caused by their northerly habitat.

Large differences in reproduction and growth may warrant the separation of the population in three localities. The existence of subpopulations was also suggested in southeastern Pacific Ocean by Litvinov (1990). However, more specimens exhibited by time series are needed to confirm the independence of each population. It is also needed to refine reading methods of rings and growth pattern analysis.

**Acknowledgements.** - We thank O. Baba, H. Kono, T. Iida, Y. Kamiguchi, Y. Kurata, S. Yamaguchi, T. Yamakawa and the crew of Kaiyo-maru for their cooperation in collecting specimens. This study was supported partly by a grant in Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture in Japan (No.07456086).

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**REPRODUCTIVE MODE OF THE TAWNY NURSE SHARK,  
*NEBRIUS FERRUGINEUS*  
(ELASMOBRANCHII: GINGLYMOSTOMATIDAE)  
IN OKINAWA WATERS, WITH COMMENTS ON  
INDIVIDUALS LACKING THE SECOND DORSAL FIN**

by

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Senzo UCHIDA (2) & Mamoru TAMAKI (3)

In August 1995, a gravid female of the tawny nurse shark, *Nebrius ferrugineus* (Lesson, 1830) (= *N. concolor* Rüppell, 1837) was caught off Ishigaki Island in the Okinawa archipelago. The right ovary had numerous mature eggs measuring 25-30 mm diameter (Fig. 1A). The left uterus of this female contained two embryos, female 33.8 cm TL and male 29.7 cm TL, and an egg capsule (Fig. 1B). No trace of yolk (umbilical) stalk was found on its abdomen and no placental connection was formed. The abdomen of the 33.8 cm TL embryo was quite distended and that of the smaller embryo was slender (Fig. 1B); during embryonic development the smaller embryo might have failed to feed efficiently. These observations were reported by Teshima *et al.* (1995) who suggested that the reproductive mode for the tawny nurse shark is oophagy. Furthermore, the ovary with numerous mature ova for this species resembled that of an oophagous lamnid in egg production (Gilmore, 1983; Pratt, 1988).

The Okinawa gravid female had no second dorsal fin, but the embryos were normal. Teng (1958) was the first to report the lack of the second dorsal fin in *N. ferrugineus*. Descriptions of individuals lacking the second dorsal fin were given by Yoshino *et al.* (1981), Yanagisawa (1983), and Taniuchi and Yanagisawa (1987).

In 1995-1996, twelve other specimens of *N. ferrugineus* were caught with longlines, also in the Okinawa waters, around Ishigaki, Iriomote and Miyako islands (Fig. 1C, D, E). Six of them were found missing the second dorsal fin (Table I) with five caught near Ishigaki island. Thus, with the previous records, a total of 13 tawny nurse sharks have been reported lacking their second dorsal fin (Table I), with 11 found in the waters of southwestern Japan (Fig. 2) from Miyako Island to northern Taiwan. The remaining two specimens were caught in waters off Wakayama Prefecture in 1981 and 1986, about 1,500 km far northeast of the Ishigaki Island. These two specimens might originate from Okinawa and could have used the Kuroshio Current to migrate from the subtropical Okinawa waters northward at a period when this current was stronger (Yanagisawa, 1983).

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Abnormal tawny nurse sharks seem not rare in the Okinawa waters. Indeed, fishermen of Yaeyama arees (Ishigaki and Iriomote islands) reported us that tawny nurse sharks with a single dorsal fin are regularly encountered in coral reef areas where they dive for octopus. The tawny nurse shark is a "competitor" for these fishermen who called it the "octopus-eater".

**RÉSUMÉ.** - Mode de reproduction du requin-nourrice fauve, *Nebrius ferrugineus* (Elasmobranchii: Ginglymostomatidae) dans les eaux d'Okinawa, et remarques sur des individus dépourvus de seconde nageoire dorsale.

En août 1995, une femelle gravide de requin-nourrice fauve, *Nebrius ferrugineus* (Lesson, 1830) (= *N. concolor* Rüppel, 1837) a été capturée au large de l'île d'Ishigaki dans l'archipel d'Okinawa. L'ovaire droit contenait de nombreux oeufs matures de 25 à 30 mm de diamètre (Fig. 1A). L'utérus gauche abritait deux embryons, une femelle de 33,8 cm LT et un mâle de 29,7 cm LT, ainsi qu'une capsule ovigère (Fig. 1B). L'abdomen de l'embryon de 33,8 cm LT était très distendu alors que celui de l'embryon plus petit était plat. Aucune trace de cordon ombilical n'a été trouvée sur l'abdomen et aucune connection placentaire n'était formée; durant le développement embryonnaire, le plus petit embryon n'a sans doute pas réussi à se nourrir correctement. Ces observations ont été rapportées par Teshima *et al.* (1995) et elles suggèrent que le mode de développement des embryons de ce requin nourrice se fait par oophagie. De plus, l'ovaire de cette femelle portant de nombreux oeufs à maturité ressemblait à celui d'un Lamnidae oophage (Gilmore, 1983; Prat, 1988).

La femelle gravide d'Okinawa n'avait pas de seconde nageoire dorsale, mais ses deux embryons étaient normaux. Teng (1958) a été le premier à signaler l'absence de cette seconde nageoire chez *N. ferrugineus*. D'autres spécimens sans seconde nageoire dorsale ont été décrits par Yoshino *et al.* (1981), Yanagisawa (1983) et Taniuchi et Yanagisawa (1987).

En 1995-1996, douze autres spécimens de *N. ferrugineus* ont été capturés avec des palangres, également dans les eaux d'Okinawa, autour des îles Ishigaki, Iriomote et Miyako (Fig. 1C, D, E). Six d'entre eux n'avaient pas de seconde nageoire dorsale (Tableau I), et cinq provenaient de l'île Ishigaki. En incluant les données de la littérature, 13 requins-nourrices fauves ont été signalés sans seconde nageoire dorsale (Tableau I), dont 11 provenant des eaux méridionales du Japon (Fig. 2) entre l'île de Miyako et le nord de Taiwan. Les deux autres spécimens ont été capturés au large de la Préfecture de Wakayama, en 1981 et 1986, à environ 1 500 km dans le nord-est de l'île Ishigaki. Ces deux spécimens pourraient provenir d'Okinawa, en ayant utilisé le courant Kuroshio pour migrer vers le nord, à une période où ce courant était plus fort (Yanagisawa, 1983).

Les requins-nourrices fauves présentant une anomalie de leurs nageoires dorsales ne semblent pas rares dans les eaux d'Okinawa. En effet, des pêcheurs de la région de Yaeyama (îles Ishigaki et Iriomote) nous ont signalé qu'ils rencontraient régulièrement des individus sans seconde nageoire dorsale dans les zones coralliennes dans lesquelles ils plongent pour rechercher les poulpes. Le requin-nourrice fauve est un "compétiteur" pour ces pêcheurs de poulpes qui l'ont surnommé "le mangeur de poulpes".





Fig. 1. - A: Ovary of the 270 cm TL female captured off Ishigaki Island, Okinawa, on 10 August 1995. B: The two embryos and the egg capsule (Ec) found in the left uterus. C: Some sharks caught off Ishigaki Is., Okinawa, with three tawny nurse sharks without second dorsal fin: the first and third specimens from front of the picture. D: Male of 289 cm TL of tawny nurse shark, *Nebrius ferrugineus*, lacking its second dorsal fin, caught off Miyako Is., Okinawa, on 26 July 1996. E: Male of 268 cm TL without second dorsal fin, caught off Ishigaki Is., Okinawa, on 30 August 1996.

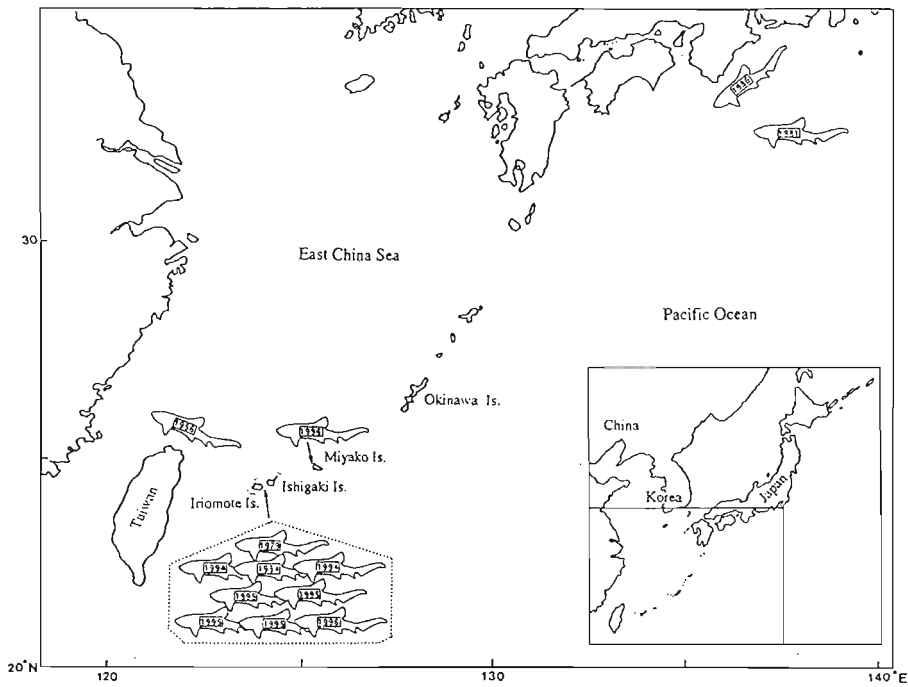


Fig. 2. - Locations of the 13 specimens of *Nebrius ferrugineus* caught in the Japanese waters without second dorsal fin, with the date of record shown in the outlined sharks.

Table I. - Specimens of tawny nurse shark, *Nebrius ferrugineus*, lacking the second dorsal fin, reported in the literature and in the present study.

Authors	Date	Locality	TL (cm)	Sex	n° LSDF / n° normal
Teng, 1958	1956	Keelung	248	M	1 / 1
Yoshino <i>et al.</i> , 1981	1979	Ishigaki	106	F	1 / 3
Yanagisawa, 1983	1981	Wakayama	282	M	1 / 1
Taniuchi and Yanagisawa, 1987	1986	Wakayama	290	M	1 / 1
Teshima <i>et al.</i> , 1995	Oct. 1994	Ishigaki	272	F gravid	1 / 2
“ “	Nov. 1994	Ishigaki	276	M	2 / 3
“ “	Nov. 1994	Ishigaki	177	F	-
Current study	7 Aug. 1995	Ishigaki	278	F	1 / 4
“ “	10 Aug. 1995	Ishigaki	237	M	2 / 4
“ “	10 Aug. 1995	Ishigaki	238	M	-
“ “	20 Aug. 1995	Ishigaki	297	M	1 / 2
“ “	26 Jul. 1996	Miyako	289	M	1
“ “	30 Aug. 1996	Ishigaki	268	M	1

Key-words. - Tawny nurse shark, *Nebrius ferrugineus*, INW, Okinawa, Reproduction, Oophagy, Abnormality, Dorsal fins.

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## CAPTURE OF A MATURE FEMALE MEGAMOUTH SHARK, *MEGACHASMA PELAGIOS*, FROM MIE, JAPAN

by

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Osamu TSUKADA (4) & Masami FURUTA (4)

**ABSTRACT.** - A mature female megamouth shark, *Megachasma pelagios*, was collected by purse seining 12 nautical miles south from Mikizaki, Owase City (33°46'N, 136°16'E) at 11:40 P.M., 30 April, 1997. The specimen was caught between the surface and 150 m. This is the tenth record of a megamouth shark and the largest specimen of this species yet recorded. The total length is 5.44 m and the weight is 1,040 kg. The external morphology and measurements as well as the anatomy of selected viscera are described. The pharyngeal region has processes, 10-20 mm in length, covered with tridentate dermal denticles. The processes of the region are described for the first time from the present specimen. The total number of tooth rows are 55 in the upper jaw and 75 in the lower jaw. The vertebral numbers are 151 in total, 64 in precaudal, and 87 in caudal. The ileum has a ring-type spiral valve with 23 turns. The stomach contained a dark-red chyme with many fragments of carapaces, eyes, and eye stalks of euphausiids. Mating scars were observed on the trunk, head, and first dorsal fin. The right ovary possessed a large number of whitish yellow oocytes, about 5-10 mm in diameter. The expanded uteri measured 260 mm in width. The specimen was mature.

**RÉSUMÉ.** - Capture d'une femelle mature de requin grande gueule, *Megachasma pelagios*, au large de Mie, Japon.

Le 30 avril 1997, à 23h40, une femelle mature de requin grande gueule a été capturée à 12 milles nautiques au sud de Mikizaki, Owase (33°46'N, 136°16'E) à une profondeur comprise entre la surface et 150 m. Sa longueur totale était de 5,44 m pour un poids de 1 040 kg; ce spécimen est le 10ème et le plus grand des requins grande gueule signalés à ce jour. Sa morphologie externe est décrite, ainsi que l'anatomie de certains organes internes. Sa région pharyngienne porte des papilles d'une longueur de 10 à 20 mm, et est recouverte de denticules cutanés tricuspidés. Ces papilles sont décrites ici pour la première fois. La mâchoire supérieure porte 55 rangées de dents et l'inférieure 75. Le nombre total de vertèbres est de 151, dont 64 précaudales et 87 caudales. La valvule spirale, annulaire, compte 23 spires. Le contenu stomacal était constitué d'une bouillie rouge foncé contenant de nombreux fragments de carapaces, des yeux et des pédoncules oculaires d'euphausiacés. Ce spécimen présentait des cicatrices d'accouplement sur le tronc, la tête et sur la première nageoire dorsale. L'ovaire droit contenait un grand nombre d'ovocytes jaunâtres d'un diamètre de 5 à 10 mm. La largeur de l'utérus dilaté atteignait 260 mm.

**Key-words.** - *Megachasma pelagios*, Megamouth shark, INW, Japan, Mikizaki, Morphology, Mature female, Mating scar, Record.

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The first megamouth shark was a 4.46 m TL male captured on 15 November 1976 from 42 km northeast of Oahu, Hawaii by naval research vessel AFB-14 (Taylor *et al.*, 1983). The second specimen, 4.49 m TL mature male, was captured off California in November 1984 (Lavenberg and Seigel, 1985). The third specimen, 5.15 m TL mature male, was recorded in August 1988 from western Australia (Berra and Hutchins, 1990). The fourth megamouth shark, ca. 4 m TL mature male, was stranded on a beach in Hamamatsu, Japan in January 1989 (Nakaya, 1989). The fifth specimen, ca. 4.9 m TL, was caught off Yaizu, Japan in June 1989 (Miya *et al.*, 1992). The sixth specimen, 4.9 m TL mature male, was caught off California in October 1990 and was released alive for a telemetric study (Nelson *et al.*, 1997). The seventh specimen, 4.71 m TL, was stranded on a tidal flat near Gannosu, Fukuoka, Japan in November, 1994 and it was the first female of the species examined by scientists (Takada *et al.*, 1997b). The eighth specimen, ca. 1.8 m TL immature male, was captured off Senegal on May 1995 (Séret, 1995). The ninth specimen, 1.9 m TL immature male, was caught in Brazilian waters in September 1995 (Amorim *et al.*, 1995) (Fig. 1).

A mature female megamouth shark, *Megachasma pelagios*, was captured from Mikizaki, Mie, Japan. This is the tenth record of a megamouth shark. This study describes the capture and its external morphology, viscera, and the stomach and intestinal contents.

## MATERIAL AND METHODS

The specimen was a mature female collected by purse seining (ring net) from Mikizaki, Owase City, on 30 April, 1997. The fishing vessel (Seiyo Maru n°1) was 135 tons and 37 m in length. The purse seine net (knotless net) was 255 m in height, 1,200 m in circumference, and about 382 m in diameter when encircles the fish with netting gear. The material of the net was polyester (Tetron) with about 1.4 cm in mesh size.

The specimen was carried by conveyance vessel (Seiyo Maru n°38, 260 tons, 43 m) to the Nayaura Port and it arrived early morning of 1 May, 1997. The freshly dead specimen was carried into the freezing warehouse and the freezing process was started immediately at -30°C. The specimen was frozen from 1 May to 1 June, 1997. The body was covered with plastic sheets to protect it from direct contact with the chilling air.

Thawing required 11 days, from 12:00 P.M., 1 June to 7:00 A.M., 12 June at the refrigerator warehouse. The temperature of the refrigerator was about 3 to 5°C (Fig. 2). The body temperature (musculature 20 cm under the skin) of the specimen was measured at the trunk below the 1st dorsal fin base that recorded at least 2 times per day (morning and afternoon) (Fig. 2). On 1 June, the body temperature was -28.7°C. From 2 to 12 June, the body temperature increased by about 1°C daily for three days and 0.1-0.5°C for the other days. The body temperature was held at about -3 to -5°C until dissection of the shark. During the thawing process, little wrinkles appeared on the body surface due to dehydration, but no other substantial effect has been observed. It was confirmed that the internal organ and muscles were thawed uniformly and with a high degree of freshness.

Takada *et al.* (1997a) reported that thawing of the megamouth shark n°7 from Fukuoka required nine days. Their frozen specimen was suspended on a stretcher in the half-strength sea water tank during thawing. The Fukuoka megamouth shark thawing method and our thawing method seemed to be successful.

The external morphology and measurements as well as the anatomy of selected viscera and the contents of the stomach and intestine were examined. Measurements follow Yano and Musick (1992), with addition of the some measurements of Compagno (1984).

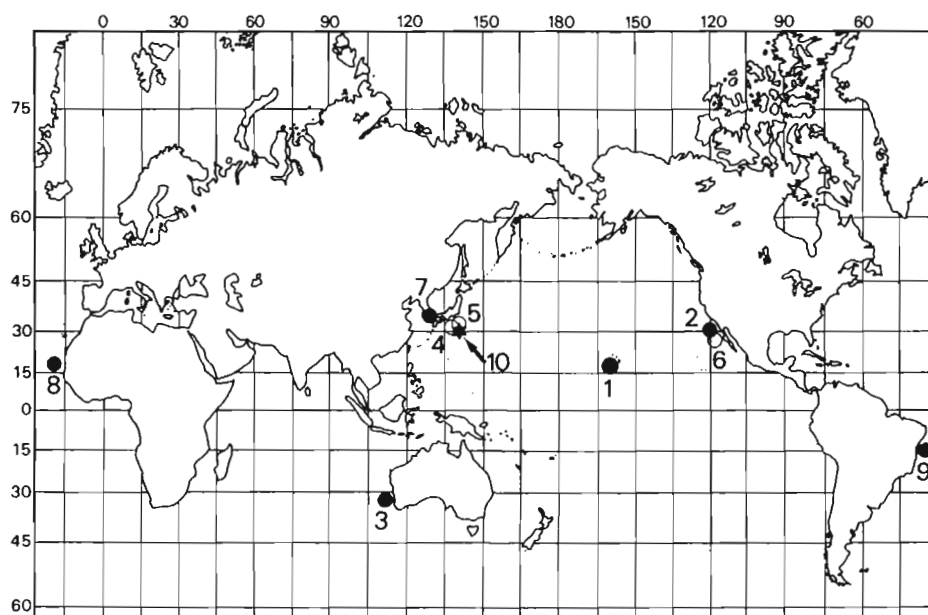


Fig. 1. - Records of the *Megachasma pelagios* (n°1-10) from the world. ★: Present specimen; ●: Specimens were collected; ○: Specimens were released or only photographed.

## RESULTS AND DISCUSSION

### Locality and depth preference

The specimen collected by commercial purse seining of mackerel at 12 nautical miles south from Mikizaki (33°46'N, 136°16'E), Owase City, Mie, Japan (Fig. 3). The megamouth shark was captured by fishing vessel at night, 11:40 P.M., of 30 April, 1997 with five tons of mackerel (*Scomber japonicus*). This finding suggests that megamouth sharks swim with *S. japonicus* and feed on the same prey animals (i.e., euphausiid shrimps). The net was set from the surface to 150 m where the sea was more than 1,500 m deep. Sea condition was calm and the surface water temperature was 18.1°C. The current was 0.5 knot to the north.

The first specimen of megamouth shark entangled two large parachutes as sea anchors at a depth of about 165 m in water with a bottom depth of approximately 4,600 m (Taylor *et al.*, 1983). The second specimen was collected by a commercial drift net operating off the Californian coast at a depth of 38 m (Lavenberg and Seigel, 1985). The fifth megamouth shark was captured by set net between surface and 40 m depth in Suruga Bay, Japan (Miya *et al.*, 1992). Miya *et al.* (1992) reported that nearly every morning the fishermen hauled the trap net to examine their catches. The megamouth shark was found in their catches along with coastal fishes (*Engraulis japonicus*, *Trachurus japonicus* and *Scomber japonicus*). The sixth specimen was captured by drift nets just past midnight off Dana Point in the California, and it was entangled the nets at a depth of about 23 m (Lavenberg, 1991). The eighth specimen was caught by French tuna purse seine off Senegal (Séret, 1995). The ninth specimens was caught by tuna longlines (Amorim *et al.*, 1995). The setting depth of tuna longlines is usually shallower than about 200 m (Yano

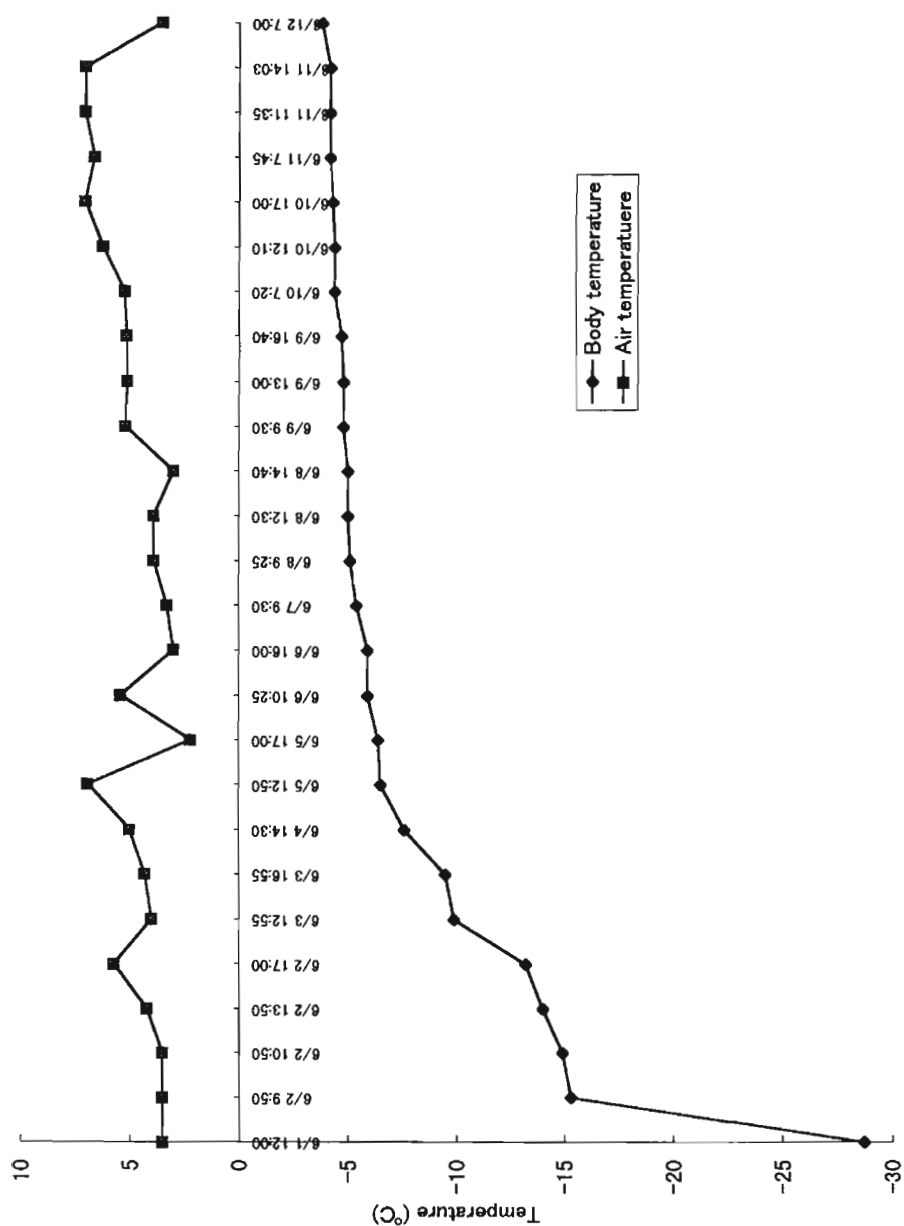


Fig. 2. - Variation of the body temperature of the *Megachasma pelagios* and the air temperature of the warehouse during thawing process.





Fig. 3. - The megamouth shark, *Megachasma pelagios*, collected by commercial purse seine at 12 miles south from Mikizaki, Mie, Japan.

and Abe, 1998). The capture depths of all these megamouth sharks are recorded from the surface to 200 m. In the ultrasonic telemetry of the sixth specimen, the shark had spent the daylight hours at a depth of 120-166 m ( $\bar{x} = 149$  m), and at dusk had ascended to around 12-25 m ( $\bar{x} = 17$ ) below the surface where it remained throughout the night (Nelson *et al.*, 1997). The capture depths and swimming depths recorded by telemetry have suggested that the megamouth shark usually swims near the surface at nighttime and in mid-water (shallower than 200 m) at daytime. Hutchins (1992) stated that this vertical migration is obviously triggered by light changes, but may also be a response to the movement of the planktonic animals on which it feeds. Nelson *et al.* (1997) reported that the four twilight depth-change events were distinct and always spanned the times of sunset or sunrise. They suggested that the depth chosen by the megamouth sharks was to a large degree determined by light levels.

#### Measurements and morphology

Measurements of the specimen are given in table 1. The total length is 5.44 m and the body weight is 1,040 kg. Body is tadpole-like with larger head and tapering trunk and tail. Snout is short and broadly rounded in dorsal view. Mouth is terminal and large. Body is blackish brown dorsally, gradually becoming paler ventrally, abruptly white below the level of the pectoral and pelvic fins. The mouth roof is silvery on the dorsal and lateral parts, oral membrane silvery. The "tongue" (hyoid) is purplish brown with slight silvery tint dorsally and ventrally. A narrow transverse bright white band is present just above the black area of the upper jaw between the right and left nostrils. The distal webs of upper surfaces of pectoral and pelvic fins have conspicuous dark wavy lines, often parallel, which are channels of bare skin between areas of denticulate skin.

Table I. - Measurements of a female megamouth shark, *Megachasma pelagios*, from Mie, Japan.  
 \*: methods of measurements follow Compagno (1984).

Measurements	(cm)	% of TL	Measurements	(cm)	% of TL
<b>Total length</b>	544		<b>Eye:</b> horizontal diameter	6	1.10
<b>Snout tip to:</b>			vertical diameter	5.5	1.01
outer nostrils	26	4.78	<b>Interorbital width</b>	63	11.58
eye	46	8.46	<b>1st dorsal fin:</b> overall length	55	10.11
spiracle	70	12.87	length base	45	8.27
mouth			length ant. margin*	46	8.46
1st gill opening	113	20.77	length post. margin	32	5.88
2nd gill opening	123	22.61	length inner margin*	9	1.65
3rd gill opening	136	25.00	height	27	4.96
4th gill opening	147	27.02	<b>2nd dorsal fin:</b> overall length	37.5	6.89
5th gill opening	156	28.68	length base	30	5.51
pectoral origin	157	28.86	length ant. margin*	27	4.96
pectoral axilla	183	33.64	length post. margin	16	2.94
pectoral end when laid back	263	48.35	length inner margin*	8.5	1.56
pelvic origin	304	55.88	height	14	2.57
pelvic axilla	317	58.27	<b>Anal fin:</b> overall length	21	3.86
cloaca	306	56.25	length base	14	2.57
1st dorsal origin	186	34.19	length post. margin	11	2.02
1st dorsal axilla	239	43.93	height	10	1.84
2nd dorsal origin	297	54.60	<b>Pectoral fin:</b> length base	37	6.80
2nd dorsal axilla	326	59.93	length ant. margin	106	19.49
anal fin origin	342	62.87	length distal margin	24	4.41
anal fin axilla	358	65.81	length post. margin	79	14.52
upper caudal origin	382	70.22	height*	94	17.28
lower caudal origin	383	70.40	<b>Pelvic fin:</b> overall length	38	6.99
<b>Distance between bases:</b>			length base	26	4.78
1st and 2nd dorsal	58	10.66	length ant. margin	37	6.80
2nd dorsal and caudal	54	9.93	length post. margin*	29	5.33
pectoral and pelvic	122	22.43	length distal margin	10	1.84
pelvic and anal	30	5.51	<b>Caudal fin:</b> length dorsal lobe	166	30.51
anal and caudal	25	4.60	length ventral lobe	78	14.34
<b>Distance between insertions:</b>			dorsal tip to notch	13	2.39
pectoral and pelvic	148	27.21	depth notch	7	1.29
<b>Nostrils:</b>			length terminal dorsal lobe*	17	3.13
distance between inner corners	45	8.27	length upper postventral caudal margin*	120	22.06
<b>Prenarial length*</b>	34	6.25	length lower postventral caudal margin*	45	8.27
<b>Mouth:</b> width	70	12.87	caudal fork length*	60	11.03
length	64	11.76	caudal fork width*	55	10.11
<b>Gill opening lengths:</b> 1st	25	4.60	<b>Head height*</b>	84	15.44
2nd	25	4.60	<b>Trunk height*</b>	82	15.07
3rd	27	4.96	<b>Abdomen height*</b>	70	12.87
4th	24	4.41	<b>Tail height*</b>	48	8.82
5th	20	3.68	<b>Caudal peduncle height*</b>	29	5.33
<b>Spiracle</b>	2	0.37			

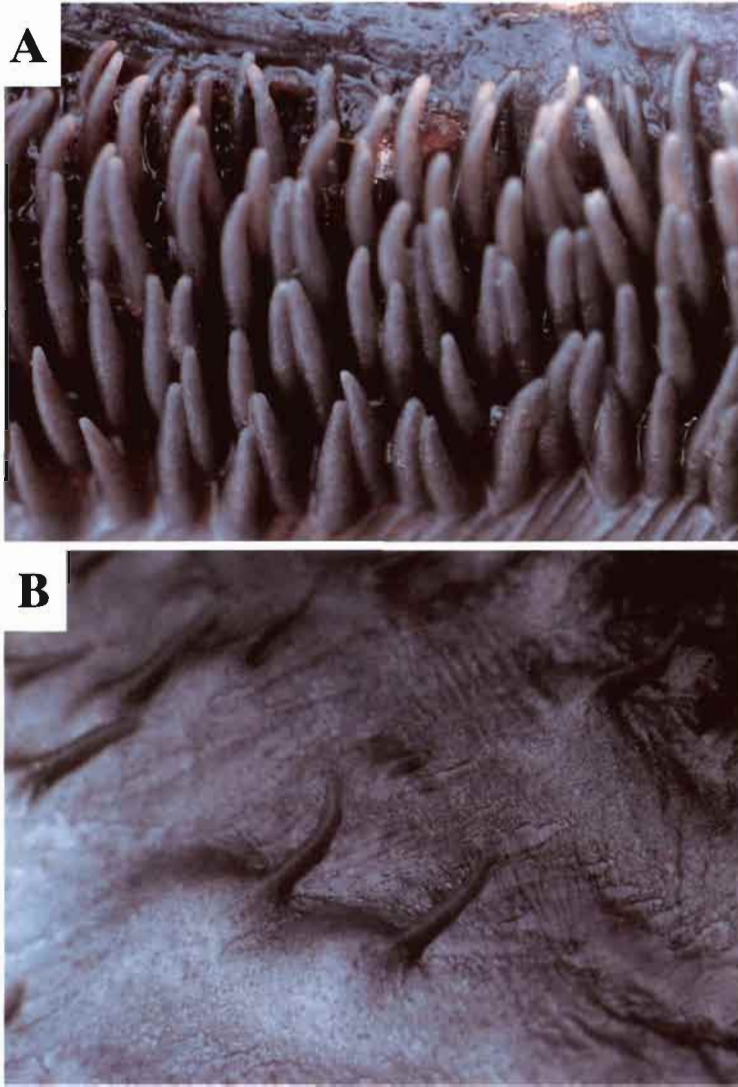


Fig. 4. - Gill raker papillae (A) and pharyngeal processes (B) of the megamouth shark, *Megachasma pelagios*.

The external morphology and coloration of this specimen are similar to other specimens described in detail by Taylor *et al.* (1983) and Nakaya *et al.* (1997). A circular crater-like wound was found on the abdomen between the pectoral and pelvic fins. The size of the wound was 52.4 mm x 32.1 mm. This wound could have been caused by a cookie-cutter shark, *Isistius brasiliensis*. Among the other specimens of the megamouth shark, similar wounds by cookie-cutter shark were reported on the specimen n°1 from Hawaii (Taylor *et al.*, 1983), specimen n°3 from Western Australia (Berra and Huchins, 1990, 1991) and specimen n°7 from Fukuoka, Japan (Yamaguchi and Nakaya, 1997).

Teeth are small (about 5 mm in height) and numerous. The lower teeth are larger than the upper ones. The shape of the teeth of this specimen is similar to the shape of the megamouth n°7 teeth described by Yabumoto *et al.* (1997). There are 27 rows in the right upper jaw, 28 rows in the left upper jaw, 37 rows in the right lower jaw and 38 rows in the left lower jaw. The total number of tooth rows is 55 in the upper jaw and 75 in the lower jaw. There are four functional teeth on each jaw. Average width between teeth of the right upper jaw is 11.43 mm (8.00-19.30 mm, SD = 2.684) and the right lower jaw is 11.14 mm (7.30-18.30 mm, SD = 2.720). The symphyseal toothless spaces are 190 mm in the upper jaw and 70 mm in lower jaw. The number of tooth rows in the mature female specimen are fewer than those in the male and the immature female. The male holotype has 108 tooth rows in the upper jaw and 128 in the lower jaw (Taylor *et al.*, 1983), and the immature female from Fukuoka has 83 tooth rows in the upper jaw and 97 in the lower jaw (Yabumoto *et al.*, 1997).

Dermal denticles on the trunk, abdomen, head, and fin regions are small (about 0.5 mm in crown length) and flattened, giving the skin a smooth texture. Mucous denticles on the rear and center of the palate are loosely spaced, not closely imbricated. The shapes of the dermal and mucous denticles of this specimen are similar to the shape of the megamouth n°7 described by Yano *et al.* (1997a).

#### Gill raker and pharyngeal process

Gill raker papillae are small, about 10-20 mm in length, densely packed, slender, and tapering to a blunt point (Fig. 4A). Dermal denticles of the gill rakers are closely imbricated, leaf-shaped and tridentate, with the tips of the denticles pointing toward to the buccal cavity. The shape of the gill raker of our specimen is the same as in the specimens n°1 and n°7 described by Taylor *et al.* (1983) and Yano *et al.* (1997a). The pharyngeal region has processes, 10-20 mm in length (Fig. 4B), covered with dermal denticles. The denticles are closely imbricated, leaf-shaped and tridentate, and their shape is similar to the denticles of the gill rakers. In the present report, the processes of the pharyngeal region are the first described for the megamouth shark. We think that these processes are used for the feeding in the same way as the gill rakers.

Table II. - Weight and percent of body weight (BW) for selected viscera of the megamouth shark, *Megachasma pelagios*, collected from Mie, Japan.

Viscera	Weight (g)	Percent of BW
Liver	26650	2.563
Gall bladder	1000	0.096
Stomach	6150	0.591
Intestine	8750	0.841
Spleen	1350	0.130
Pancreas	300	0.029
Rectal gland	150	0.014
Heart	1100	0.106
Ovary	2150	0.207
Epigonal organ	1350	0.096
Uterus (left)	7950	0.764
Uterus (right)	8150	0.784

### Viscera and vertebral numbers

The weight of each organs and these percent of the body weight are given in table II. The shape and color patterns of the viscera of this specimen are similar to those of megamouth shark n°7 described in detail by Yano *et al.* (1997b). The liver is the largest organ and the ratio is 2.6% of the body weight. It is smaller than that (4.2% of body weight) of the immature female specimen from Fukuoka, Japan, reported by Yano *et al.* (1997). The ileum has a ring-type spiral valve with 23 turns. The number of turns of spiral valve is almost the same as the number of turns (24) of the Fukuoka megamouth (Yano *et al.*, 1997b).

The ovary is found on the right side at the anterior end of the body cavity (Fig. 5B). The epigonal organ is found on the left side at the anterior end of the body cavity. Both the ovary and the epigonal organ (1,350 g) are attached by the mesovarium to the body wall and ostium. The ovary is 410 mm long and 220 mm wide, and its weight is 2,150 g. On its dorsal surface there is a funnel-like fold (59.5 mm in diameter) which connects into the lumen of the ovary through several openings. The ovary possess a large number of whitish yellow oocytes containing yolk, about 5-10 mm in diameter (Fig. 5A). Castro *et al.* (1997) reported that the oocytes of the megamouth shark n°7 measured 1.1-2.7 mm. Tanaka and Yano (1997) observed histologically that a 600  $\mu$ m oocyte contained a large number of very fine yolk granules. They suggested that this specimen was close to maturity. The type of ovary suggests that megamouth embryos are oophagous (Castro *et al.*, 1997; Tanaka and Yano, 1997).

The ostium is developed and measures 52 mm x 28 mm in size. The nidamental glands are developed and are 32 mm (width) x 75 mm (length) in left and 35 mm x 85 mm in right. The expanded uteri measure 260 mm (width) x 745 mm (length) in left and 260 x 790 mm in right (Fig. 5A). Castro *et al.* (1997) reported that the nidamental glands of megamouth shark n°7 were poorly developed, 22 mm in width, and the flaccid uteri measured 110 mm in width.

The reproductive organs of the specimen are mature. Because the ovary has whitish yellow oocytes containing yolk, developed nidamental glands, and expanded uteri. The basic structure and shape of the reproductive organs are similar to the organs of the immature specimen of the megamouth shark reported by Castro *et al.* (1997).

The vertebral numbers are 151 in total, 64 in precaudal, and 87 in caudal. Yano *et al.* (1997c) reported vertebral number of the megamouth shark observed by X-ray, and they could count 125 vertebrae from the tip of the dorsal lobe of the caudal fin to beyond the origin of the first dorsal fin with certainty. The caudal vertebral number was 82 in their report but they reported that there were difficulties to examine all vertebrae in large megamouth sharks via X-radiography. The total vertebral number of the megamouth shark in the present study is the first certain count for this species.

### Stomach and intestinal contents

The stomach contents consisted of dark-red chyme containing many fragments of carapaces, eyes, and eye stalks of euphausiids. We could not identify the euphausiid contents to the species level. The stomach was relatively full of chyme and weighted 14,950 g (1.44% of body weight). The intestinal contents were reddish in color and weighted 1,450 g (0.14% of body weight).

Taylor *et al.* (1983) reported that the stomach contents of the first megamouth specimen were a thick, reddish soup containing the euphausiid shrimp, *Thysanopoda*

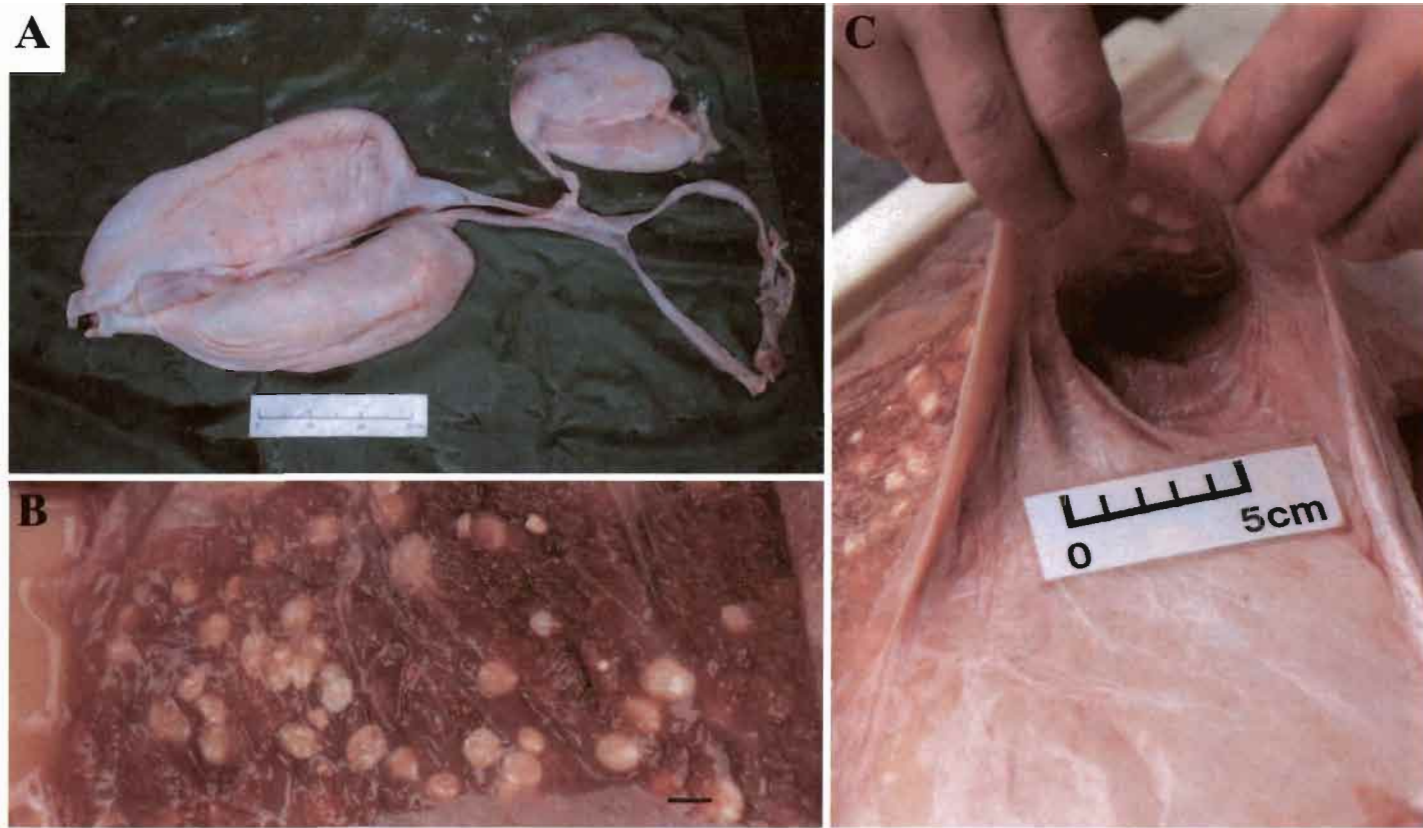


Fig. 5. - Mature female megamouth shark, *Megachasma pelagios*. A: Reproductive tract, scale bar = 300 mm. B: the ova in the ovary, scale bar = 10 mm. C: Funnel-like fold of the ovary, scale bar = 50 mm.

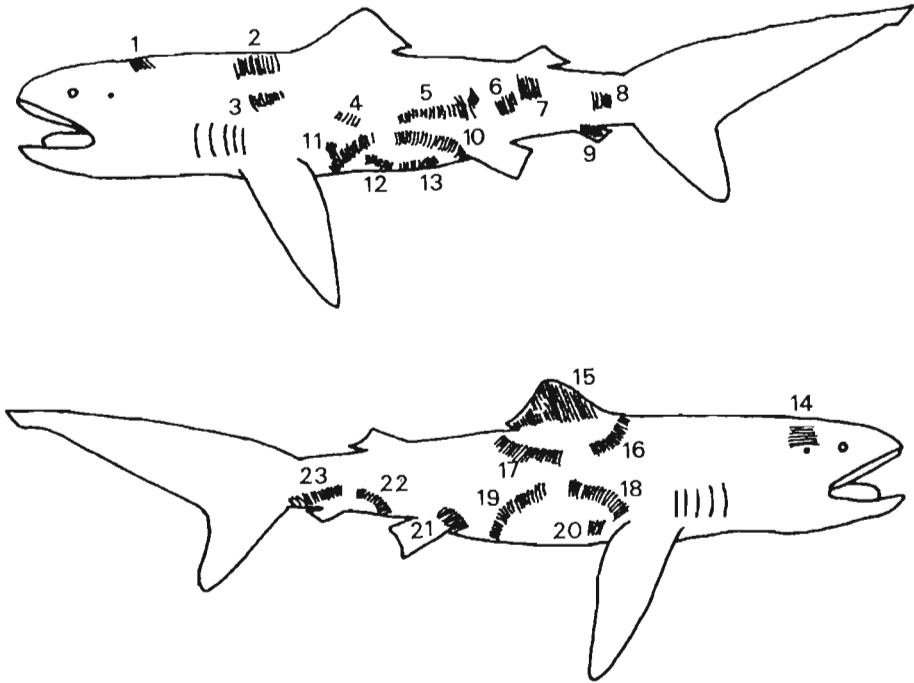


Fig. 6. - Position of twenty-three mating scars on the mature female megamouth shark *Megachasma pelagios*.

*pectinata*. The stomach contents of the second specimen included fragments of euphausiids, copepods, and the jellyfish *Atolla vanhaeffeni* (Berra and Hutchins, 1990). Yano *et al.* (1997b) suggested that the euphausiids found in the megamouth shark n°7 from Fukuoka, Japan were *Euphausia nana*. The stomach contents of the present specimen are similar to the stomach contents of the Fukuoka specimen and are probably closely related species of Euphausiids (*E. nana*) of Fukuoka specimen. Compagno (1990) suggested that the megamouth shark probably swam slowly through aggregations of euphausiids and other prey with its mouth open.

#### Mating scars

Twenty-three mating scars are found on the trunk, head, and first dorsal fin (Figs 6, 7). Fresh tooth slashes are appeared as parallel straight or curved cuts (Fig. 6). Size of the scars, number of the slashes, and width between slashes (i.e., width between teeth male shark) are shown in table III. Average width between slashes are 5.75-9.94 mm. There are slightly narrower than the space between teeth of the present specimen (11.43 mm in the upper jaw and 11.14 mm in the lower jaw). However, we think that the space between teeth of smaller individuals of male megamouth shark are probably narrower than the present female, because the number of teeth in males is larger than that in females (Yabumoto *et al.*, 1997). Mating scars by males were often found on the bodies of mature female shark in other species. Thus, we think that the tooth slashes on the female specimen are mating scars by males. Fresh semi-circular jaw impressions showing continuous tooth marks are observed on the abdomen between pectoral and pelvic fins



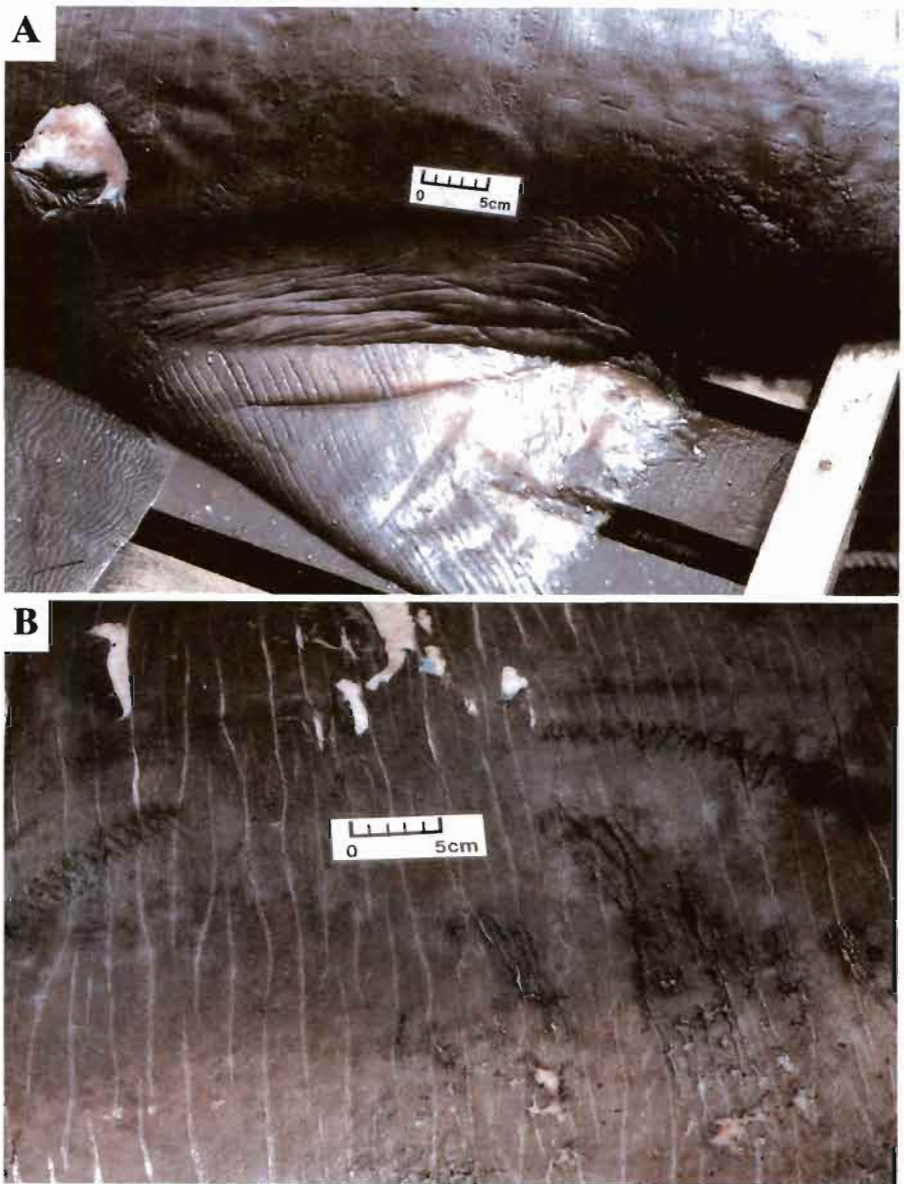


Fig. 7. - Mating scars of the megamouth shark, *Megachasma pelagios*. A: First dorsal fin (n°15 in Fig. 6); B: Abdomen between pectoral and pelvic fins (n°19, 18, 20 in Fig. 6).

(n°10, 11, 18, 19 in Fig. 6), the left and right lateral trunk below the first dorsal fin (n°4, 5, 16, 17 in Fig. 6), and the right lateral trunk between the pelvic and caudal fins (n°22, 23 in Fig. 6). No mark spaces were found between the semi-circular jaw impressions (Fig. 7). The spaces between marks are 78.2 mm in n°4 and 5; 78 mm in n°10 and 11;



300 mm in n°16 and 17; 215 mm in n°18 and 19, and 80 mm in n°22 and 23. Both jaw of the megamouth shark have symphyseal toothless space (190 mm in upper jaw and 70 mm in lower jaw). Yabumoto *et al.* (1997) reported that the symphyseal toothless space of the megamouth shark n°7 is 180 mm in upper jaw and 80 mm in lower jaw. We think that the semi-circular marks (n°4, 5, 10, 11, 22, 23) on the trunk of the mature female are mating scars by lower jaw of male megamouth sharks, and the marks (n°16, 17, 18, 19) are mating scars by upper jaw of the males.

**Acknowledgements.** - We express our sincere thanks to M. Shimizu, Seiyo Suisan, and captain and crew of "Seiyo maru n°1 and n°38", for providing the specimen of the megamouth shark. We also thank to H. Nakamura, T. Kataoka, K. Yamamoto, K. Suzuki and staff members of Toba Aquarium for their valuable cooperation during handling and dissection of the specimen.

Table III. - Sizes of wounds and slashes of mating scars of the megamouth shark, *Megachasma pelagios*, collected from Mie, Japan.

N° in Fig. 4	Width of wound (mm)	Max. length of slash (mm)	N° of slashes	Average width between slashes (mm)	Min. width between slashes (mm)	Max. width between slashes (mm)	S D
1	140.0	113.0	14	9.03	7.30	10.80	1.151
2	119.0	310.0	16	7.37	5.00	12.00	1.796
3	129.0	61.0	21	9.94	5.60	27.30	4.715
4	114.6	78.2	12	8.74	7.30	10.40	0.915
5	320.0	78.2	34	9.58	6.20	12.50	1.651
6	-	-	-	-	-	-	-
7	69.0	128.0	11	8.42	6.40	14.20	2.191
8	32.0	114.6	7	5.75	4.70	6.60	0.797
9	105.0	136.8	12	8.75	6.60	10.90	1.428
10	292.0	-	-	-	-	-	-
11	188.0	-	-	-	-	-	-
12	330.0	-	-	-	-	-	-
13	530.0	-	-	-	-	-	-
14	48.0	244.0	7	7.87	6.00	11.00	1.806
15	213.0	-	27	8.62	6.50	11.20	1.289
16	221.0	-	21	8.81	7.50	12.20	1.262
17	151.0	-	23	7.83	6.70	9.40	0.869
18	209.1	-	28	8.60	5.00	11.40	1.846
19	148.3	-	17	8.28	6.60	9.00	0.882
20	45.0	148.0	8	9.00	6.60	11.00	1.584
21	133.0	195.0	19	7.78	5.40	13.20	2.044
22	71.0	-	11	8.93	7.00	10.80	1.300
23	550.0	-	45	8.00	5.00	12.00	1.702

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## CHONDRICHTHYANS OF THE RYUKYU ISLANDS, JAPAN

by

Kazunari YANO (1)

**ABSTRACT.** - The Ryukyu Islands are located on subtropical habitats of the southern Japan. The distribution and species composition of chondrichthyans of Ryukyu Is. were studied. Thirty-four families and 110 species were recorded among which 22 families and 77 species were sharks, 10 families and 28 species were skates and rays, and 2 families and 5 species were chimaera. Juveniles of *Negaprion acutidens* and *Carcharhinus limbatus*, and mature-sized *Triaenodon obesus* and *Nebrius ferrugineus* were collected in the lagoon habitats. In the coral reef slope and off shore habitats, carcharhinid sharks, *Galeocerdo cuvier*, *Carcharhinus albimarginatus*, *C. plumbeus* and *C. brachyurus* were mainly collected. *Sphyrna lewini*, *Manta birostris* and *Aetobatus narinari* were also present in these habitats. In the epipelagic areas, the dominant species were *Prionace glauca* and *Dasyatis violacea*. *Isurus oxyrinchus*, *Alopias pelagicus*, *A. superciliosus* and *Carcharhinus falciformis* were also present in these habitats. The dominant genera in the deep sea habitats were *Squalus*, *Centrophorus*, *Etmopterus* and *Galeus*. The blackgill catshark, *Parmaturus melanobranchius*, was collected from the deep sea habitats and this is the first record from the waters around Japan. Differences in faunal ecology among locations are also discussed.

**RÉSUMÉ.** - Chondrichthyens des îles Ryukyu, Japon.

Nous avons étudié la distribution et la composition spécifique des chondrichthyens des îles Ryukyu, situées en zone subtropicale, au sud du Japon. Au total, 110 espèces de 34 familles ont été répertoriées, comprenant 77 espèces de 22 familles de requins, 28 espèces de 10 familles de raies et 5 espèces de 2 familles de chimères. Dans les lagons, des juvéniles de *Negaprion acutidens* et de *Carcharhinus limbatus* ont été capturés ainsi que des adultes de *Triaenodon obesus* et de *Nebrius ferrugineus*. Sur la pente récifale et en haute mer, les Carcharhinidae dominent: *Galeocerdo cuvier*, *Carcharhinus albimarginatus*, *C. plumbeus* et *C. brachyurus*. Dans ces mêmes habitats, *Sphyrna lewini*, *Manta birostris* et *Aetobatus narinari* sont également présentes. En zone épipelagique, les deux espèces dominantes sont *Prionace glauca* et *Dasyatis violacea*; sont aussi présentes *Isurus oxyrinchus*, *Alopias pelagicus*, *A. superciliosus* et *Carcharhinus falciformis*. En zone pélagique profonde, les genres dominants sont *Squalus*, *Centrophorus*, *Etmopterus* et *Galeus*. La holbiche à joues noires, *Parmaturus melanobranchius*, est signalée pour la première fois dans les eaux profondes du Japon. Les différences écologiques observées entre les sites étudiés sont discutées.

**Key-words.** - *Parmaturus melanobranchius*, Chondrichthyans, PSW, Japan, Ryukyu Is., Faunal ecology, First record, Inventory.

The Ryukyu Islands are located on subtropical habitats of the southern Japan. The islands develop coral reef areas. The chondrichthyan fishes of the waters around the Ryukyu Is. were reported by Uchida (1982). Yano and Kugai (1993b) reported deep-sea chondrichthyans collected from the waters around the islands by bottom longlines.

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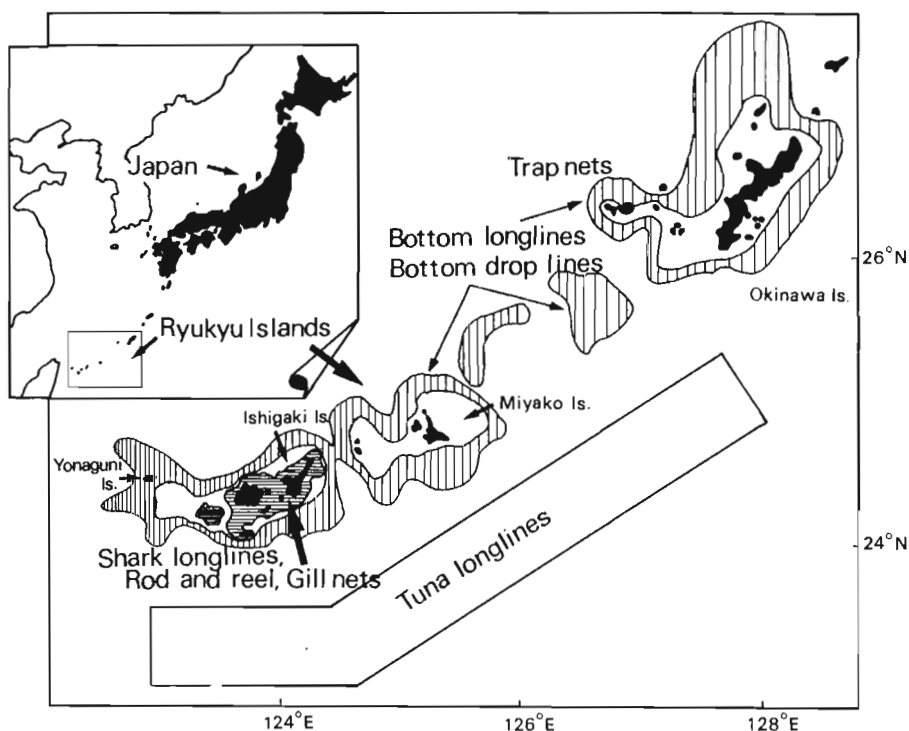


Fig. 1. - Fishing grounds of chondrichthyan fishes around Ryukyu Islands, Japan.

Nakaya and Shirai (1992) presented the fauna of deep-benthic chondrichthyans collected from the Okinawa Trough. However, the occurrence of species and the distributional patterns among the locations, lagoon habitats, coral reef slope and off shore habitats, epipelagic habitats, and deep sea habitats, were poorly known.

The purposes of this report were to summarize the occurrence of the chondrichthyans among the locations of the waters around the Ryukyu Is. In addition, it provides the first record of the blackgill catshark, *Parmaturus melanobranchius*, from the waters around the Ryukyu Is., Japan.

## MATERIALS AND METHODS

The chondrichthyans were collected from the waters around the Ryukyu Is. (Fig. 1) by bottom longline (BL), bottom drop line (vertical longline, D), shark longline (SL), tuna longline (TL), rod and reel (RR), trap net (TN), and gill net (G). The specimens were collected by several surveys of the waters around the Ryukyu Is. from 1983 to 1997. The specimens of the lagoon habitats, the coral reef slope and offshore habitats were mainly collected from the waters around the Yaeyama Is. (Ishigaki Is., Taketomi Is., Kohama Is., Aragusuku Is., Hateruma Is., and Iriomote Is.). The specimens of the epipelagic habitats were collected from the southern parts of the Okinawa Is. to the Yonaguni Is. by tuna longlines operating in the areas between 30 and 100 nautical miles (55.6-185.2 km)

from the islands. The specimens of the deep sea habitats were collected from the northern parts of the Okinawa Is. to the Yonaguni Is. by bottom longlines and bottom drop lines operating at the depth from 200 m to 1,500 m. Several species of the lagoon and the coral reef slope were observed by SCUBA diving and were photographed underwater.

The lagoon habitats are inside from reef edge, and the depths are usually shallower than 10 m. The fishing gears in the lagoon areas were mainly used by rod and reel, gill nets, and bottom drop lines. The coral reef slope and offshore habitats are outside from the reef edge. The depths of these areas are shallower than 200 m. The fishing gears in the coral reef slope and offshore habitats were mainly used by bottom longlines, bottom drop lines, shark longlines, and trap nets. The epipelagic habitats are over 30 nautical miles off shore from the islands, and depths are deeper than 2,000 m. The fishing gears in the epipelagic habitats were used by tuna longlines. The deep sea habitats are out of the coral reef slope and offshore habitats, and they are deeper than 200 m. The fishing gears in the deep sea areas were used by bottom longlines and bottom drop lines.

In addition to these data, I used list or catch information of the chondrichthyans from the waters around the Ryukyu Is. presented by Fukuda (1975), Uchida (1982, 1988), Ui *et al.* (1987), Nakaya and Shirai (1992) and Ishihara *et al.* (1997). Nakaya and Shirai (1992) reported specimens caught with bottom trawl nets from below 200 m to 1180 m at the Okinawa Trough.

Measurements follow Yano and Musick (1992). The vertebral counts were made according to the method of Springer and Garrick (1964).

Part of these specimens were deposited in the National Science Museum, Tokyo, (NSMT-P), the Kitakyushu Museum of Natural History (KMNH VR), the Natural History Museum and Institute, Chiba (CBM), and School of Marine Science and Technology, Tokai University (TMFE).

## RESULTS AND DISCUSSION

The total number of chondrichthyans of the Ryukyu Is. recorded was 34 families and 110 species among which 22 families and 77 species were sharks, 10 families and 28 species were skates and rays, and 2 families and 5 species were Chimaeroidei (Table I). In the present study, at least 73 species from 25 families were represented by 2682 specimens. The most varied composition, with many species of Squalidae and Scyliorhinidae, occurred at the deep sea habitats (Fig. 2). The secondary varied composition, with many species of Carcharhinidae, occurred at the reef slope and off shore habitats (Fig. 2). Differences in faunal ecology were found among habitats, lagoon, reef slope and off shore, epipelagic, and deep sea.

Nakaya and Shirai (1992) reported that 15 families and 37 species were recorded from the deep sea habitats of the Okinawa Trough. They stated that the Okinawa Trough has the most varied composition, with many species of Squalidae, Scyliorhinidae, and Rajidae. Uchida (1982) reported 19 families and 59 species from the waters around the Ryukyu Is. Chiu (1994) reported that 114 species of chondrichthyans were recorded from the Taiwan Strait. In the present study, the waters around the Ryukyu Is. were suggested to have a varied composition, with many species of the chondrichthyans.

Table 1. - List, number of specimens by areas, and fishing gears of the chondrichthyans collected around the Ryukyu Islands. \*: reference information, \*\*: first record from Japan, S: sight observation by SCUBA diving, P: photograph observation. Fishing gears - BL: bottom longline, D: bottom dropline, G: gillnet, RR: rod and reel, SL: shark longline, ST: stranding, T: trawl net, TL: tuna longline TN: trap net, O: others.

	Lagoon	Reef slope and off shore	Epi- pelagic	Deep sea	Fishing gears	References
<b>Chlamydoselachidae*</b>						
<i>Chlamydoselachus anguineus*</i>				+	T	Nakaya and Shirai (1992)
<b>Hexanchidae</b>						
<i>Heptranchias perlo</i>				28	BL, D	
<i>Hexanchus griseus</i>				7	BL	
<b>Squalidae</b>						
<i>Etmopterus pusillus</i>				108	BL	Nakaya and Shirai (1992)
<i>Etmopterus lucifer</i>				19	BL, D	
<i>Etmopterus brachyurus</i>				130	BL	
<i>Etmopterus molleri</i>				32	BL	
<i>Centroscyllium kamoharai*</i>				+	T	
<i>Centroscyllium owstonii</i>				4	BL	
<i>Zameus squamulosus</i>				46	BL	
<i>Somniosus longus</i>				1	BL	
<i>Isistius brasiliensis</i>				1	O	
<i>Centrophorus niaukang</i>				60	BL	
<i>Centrophorus tessellatus</i>				93	BL	
<i>Centrophorus moluccensis</i>				179	BL	
<i>Centrophorus acus</i>				268	BL, D	
<i>Centrophorus squamosus</i>				355	BL	
<i>Deania calcea</i>				29	BL	
<i>Cirrhigaleus barbifer</i>				3	BL	
<i>Squalus mitsukurii</i>				189	BL	
<i>Squalus blainvillei</i>				72	BL, D	
<i>Squalus japonicus</i>				217	BL	
<i>Squalus megalops</i>				2	BL	
<i>Squaliolus laticaudus*</i>				+	T	Nakaya and Shirai (1992)
<b>Pristiophoridae</b>						
<i>Pristiophorus japonicus</i>		1		3	BL	
<b>Squatinae*</b>						
<i>Squatina nebulosa*</i>				+	BL, T	Uchida (1982), Nakaya and Shirai (1992)
<b>Heterodontidae*</b>						
<i>Heterodontus japonicus*</i>					G	Uchida (1982)
<b>Parascyllidae*</b>						
<i>Cirrhoscyllium exipolium*</i>					TN	Uchida (1982)
<b>Orectolobidae</b>						
<i>Orectolobus japonicus</i>		12			SL	
<b>Hemiscyllidae</b>						
<i>Chiloscyllium punctatum</i>		1			BL	
<b>Stegostomatidae</b>						
<i>Stegostoma fasciatum</i>		S, P				
<b>Ginglymostomatidae</b>						
<i>Nebrius ferrugineus</i>	5	8			SL, TN	
<b>Rhincodontidae</b>						
<i>Rhincodon typus</i>		P				



Table I. - (continued)

	Lagoon	Reef slope and off shore	Epi- pelagic	Deep sea	Fishing gears	References
<b>Odontaspidae</b> <i>Odontaspis ferox</i>				5	BL	
<b>Alopiidae</b> <i>Alopias pelagicus</i> <i>Alopias superciliosus</i>			1 3		TL TL	
<b>Cetorhinidae*</b> <i>Cetorhinus maximus*</i>					ST	Uchida (1988)
<b>Lamnidae</b> <i>Carcharodon carcharias</i> <i>Isurus oxyrinchus</i> <i>Isurus paucus</i>		P	6 1		TL TL	
<b>Scyliorhinidae</b> <i>Cephaloscyllium isabellum</i> <i>Galeus nipponensis</i> <i>Galeus eastmani</i> <i>Galeus sauteri*</i> <i>Parmaturus pilosus</i> <i>Parmaturus melanobranchius**</i> <i>Apristurus platyrhynchus</i> <i>Apristurus japonicus*</i> <i>Apristurus macrorhynchus*</i> <i>Apristurus longicephalus*</i> <i>Scyliorhinus torazame*</i> <i>Scyliorhinus sp.*</i> <i>Halaelurus buergeri*</i>				25 2 128  15 7 2 + + + G TN TN	BL BL BL TN BL BL T T T G TN TN	Uchida (1982)       Nakaya and Shirai (1992) Nakaya and Shirai (1992) Nakaya and Shirai (1992) Uchida (1988) Uchida (1982) Uchida (1982)
<b>Proscylliidae</b> <i>Proscyllium venustum</i> <i>Proscyllium habereri</i>		5 43			D TN	
<b>Pseudotriakidae</b> <i>Pseudotriakis microdon</i>				22	BL	
<b>Triakidae</b> <i>Mustelus manazo</i> <i>Mustelus griseus*</i> <i>Hemistriakis japonica</i>				13  17	BL BL BL	Uchida (1982)
<b>Carcharhinidae</b> <i>Carcharhinus albimarginatus</i> <i>Carcharhinus altimus*</i> <i>Carcharhinus brachyurus</i> <i>Carcharhinus falciformis</i> <i>Carcharhinus galapagensis</i> <i>Carcharhinus leucas</i> <i>Carcharhinus limbatus</i> <i>Carcharhinus longimanus</i> <i>Carcharhinus obscurus*</i> <i>Carcharhinus plumbeus</i> <i>Carcharhinus sorrah*</i> <i>Galeocerdo cuvier</i> <i>Loxodon macrorhinus</i> <i>Negaprion acutidens</i> <i>Prionace glauca</i> <i>Triaenodon obesus</i>	11       3  1	44  2  2 1 6 S, P 24 150, S 1 5 S, P	3         156		SL SL SL TL SL SL SL, D, RR  SL SL TN SL BL SL, RR BL	Uchida (1982, 1988)          Uchida (1982, 1988)  Uchida (1982, 1988)

Table I. - (continued 2)

	Lagoon	Reef slope and off shore	Epi- pelagic	Deep sea	Fishing gears	References
<b>Sphyrnidae</b> <i>Sphyrna lewini</i> <i>Sphyrna zygaena</i>		S, P 1			SL	
<b>Pristidae *</b> <i>Pristis microdon*</i>					?	Fukuda (1975), Ui <i>et al.</i> (1987), Ishihara <i>et al.</i> (1997)
<b>Rhinobatidae</b> <i>Rhina ancylostoma</i> <i>Rhynchobatus djiddensis</i> <i>Rhinobatos schlegelii*</i>		1 4			SL SL T	Nakaya and Shirai (1992)
<b>Rajidae</b> <i>Raja kwangtungensis</i> <i>Raja macrocauda*</i> <i>Raja gigas*</i> <i>Notoraja tobitukai*</i> <i>Bathyraja isotrachys*</i>				1 + + + +	BL T T T T	Nakaya and Shirai (1992) Nakaya and Shirai (1992) Nakaya and Shirai (1992) Nakaya and Shirai (1992)
<b>Anacanthobatidae*</b> <i>Anacanthobatis borneensis*</i>				+	T	Nakaya and Shirai (1992)
<b>Dasyatidae</b> <i>Taeniura meyeni</i> <i>Dasyatis violacea</i> <i>Dasyatis sephen</i> <i>Dasyatis akajei</i> <i>Dasyatis ushieii*</i> <i>Dasyatis bennetti*</i> <i>Dasyatis kuhlii*</i> <i>Himantura uarnak*</i>	1	7	67	4	SL TL G BL TN TN TN TN	Uchida (1988) Uchida (1982, 1988) Uchida (1982, 1988) Uchida (1982, 1988)
<b>Urolophidae</b> <i>Urolophus aurantiacus*</i> <i>Urotrygon daviesi</i>				+	T BL	Nakaya and Shirai (1992)
<b>Hexatrygonidae*</b> <i>Hexatrygon longirostra*</i>				+	T	Nakaya and Shirai (1992)
<b>Rhinopteridae</b> <i>Rhinoptera javanica</i>		S, P				
<b>Myliobatidae</b> <i>Myliobatis tobijei</i> <i>Aetobatus narinari</i>		S, P S, P				
<b>Mobulidae</b> <i>Mobula japonica*</i> <i>Mobula diabolus*</i> <i>Mobula tarapacana*</i> <i>Manta birostris</i>	S, P	S, P	S		TN TN TN	Uchida (1982, 1988) Uchida (1982, 1988) Uchida (1982, 1988)
<b>Chimaeridae</b> <i>Chimaera phantasma</i> <i>Hydrolagus purpureus</i> <i>Hydrolagus mitsukurii*</i>				12 3 +	BL BL T	Nakaya and Shirai (1992)
<b>Rhinochimaeridae*</b> <i>Rhinochimaera pacifica*</i> <i>Harriotta raleighana*</i>				+	T T	Nakaya and Shirai (1992) Nakaya and Shirai (1992)
<b>Total</b>	21	318	237	2106		Overall = 2682

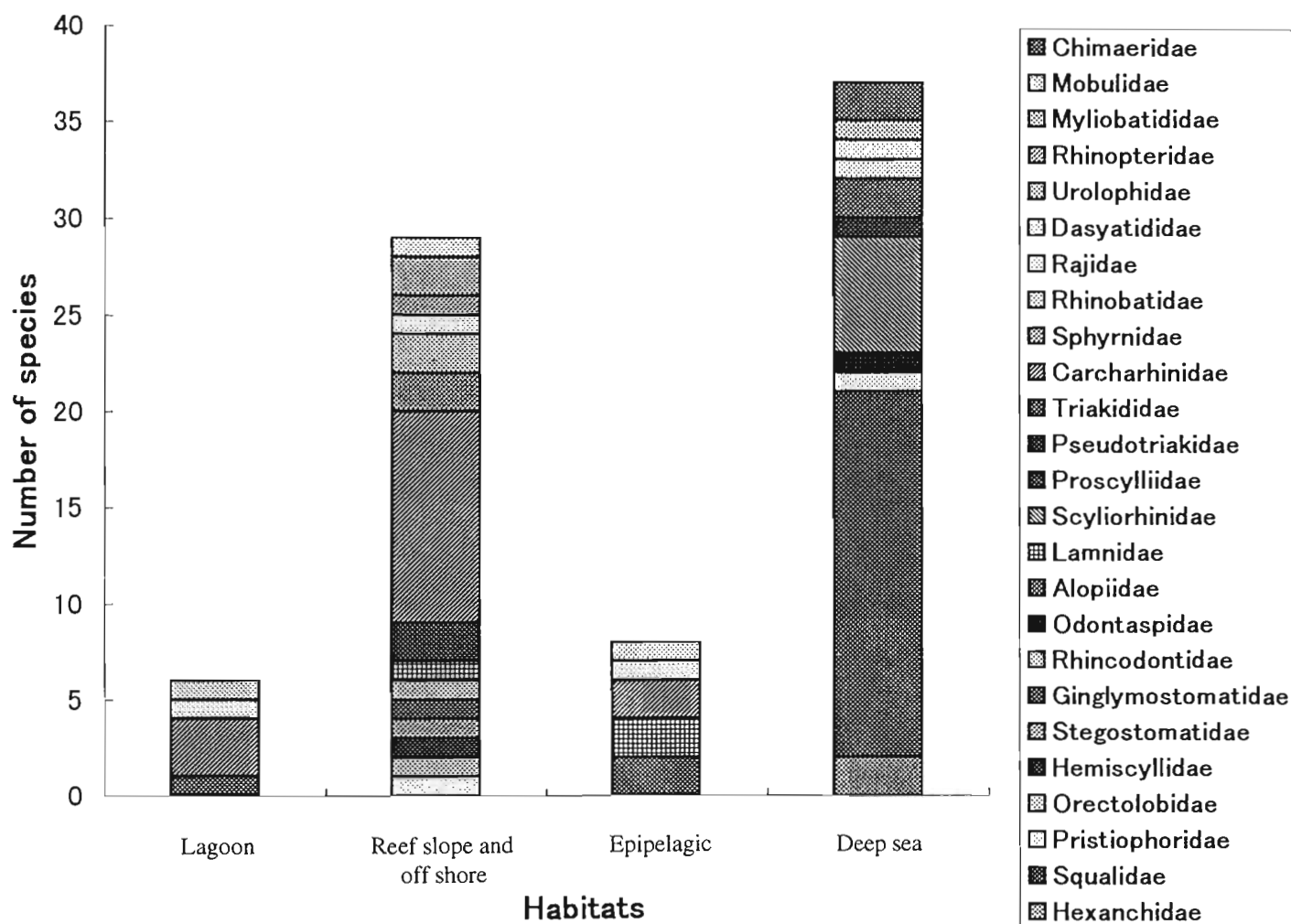


Fig. 2. - Number of species occurring in lagoonal, reef slope and off shore, epipelagic, and deep sea habitats of the Ryukyu . Japan.

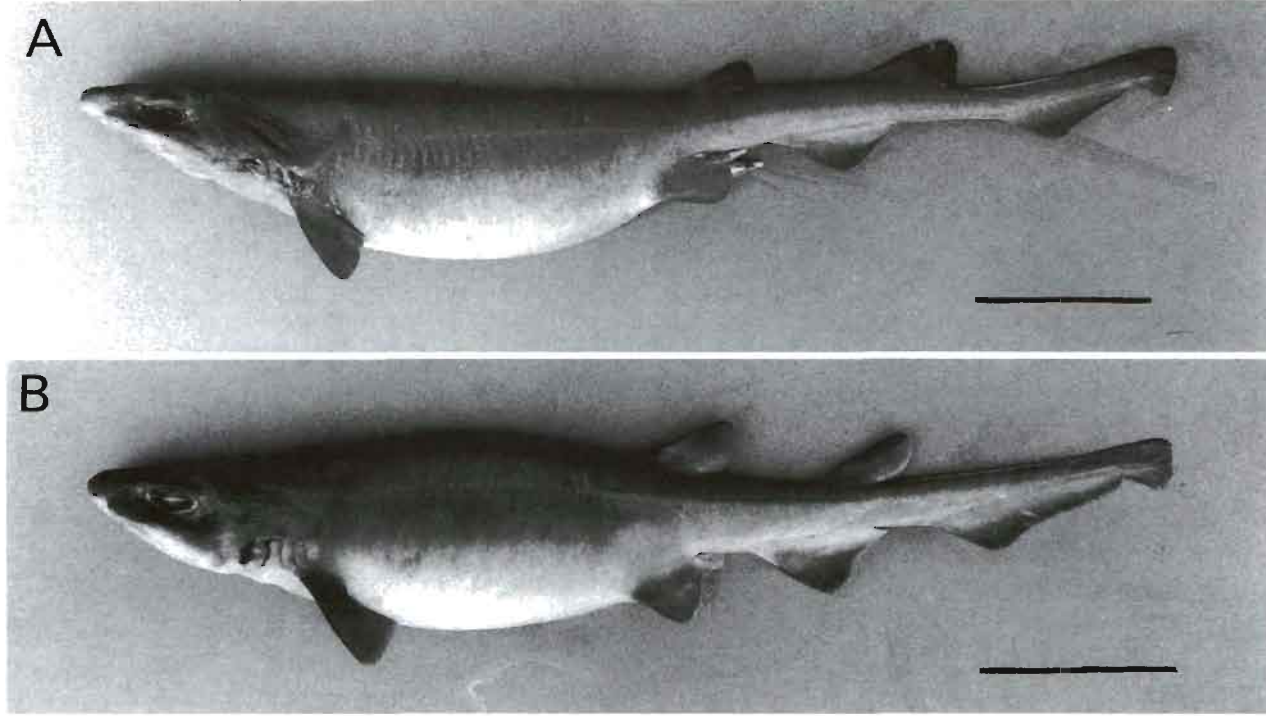


Fig. 3. - *Parmaturus melanobranchius* (A; CBM5149) and *P. pilosus* (B; KMNH VR 01314) collected off Yonaguni Island, Japan. Scales indicate 100 mm.

In the lagoon habitats, 6 species from 4 families were represented (Table I). The juveniles of *Negaprion acutidens* and *Carcharhinus limbatus* were collected in the lagoon and the mouth of rivers. However, adults of these species were collected from the coral reef slopes. I think that *C. limbatus* occurs widely in Japanese waters but is commonly misidentified as *C. melanopterus*. Therefore, it is suggested that *C. limbatus*, and not *C. melanopterus*, is the "blacktip shark" usually captured in Japanese waters. Five mature-sized specimens of *Nebrius ferrugineus* were caught at the depth of about 5 m of the habitats. Four out of 13 specimens (including specimens collected from the coral reef habitats) lacked the second dorsal fins. I observed other specimens of *N. ferrugineus* that were collected from the Kumano-nada. Specimens with the first dorsal fin only appear to be common in this species. *Manta birostris* was observed sometimes in these habitats.

In the coral reef slope and off shore habitats, 29 species from 15 families were represented (Table I). The carcharhinid sharks, *Galeocerdo cuvier*, *Carcharhinus albimarginatus*, *C. plumbeus* and *C. brachyurus*, were mainly collected from juveniles to adults. Several specimens of the species were pregnant females. *Sphyrna lewini*, *Manta birostris* and *Aetobatus narinari* were also present. Large schools (over 20 individuals) of the scalloped hammerhead shark, *S. lewini*, were observed at the Yonaguni Is. at the winter season (mainly from November to March). I observed that manta rays, *M. birostris*, migrate between the Ishigaki Is. and the Miyako Is. (about 120 km) by individual photo-identification of the black marks of the abdomen and scars of the body.

In the epipelagic habitats, 8 species from 5 families were represented (Table I). Dominant species included *Prionace glauca* and *Dasyatis violacea*. *Isurus oxyrinchus*, *Alopias pelagicus*, *A. superciliosus* and *Carcharhinus falciformis* were also present. Eighty-nine out of 109 specimens (81.7%) of *P. glauca* collected in the present study were pregnant females (186-325 cm TL,  $\bar{x}$  = 233.58 cm), and all the mature females (204-266 cm TL) had mating scars. Thirty-four males (189-279 cm TL,  $\bar{x}$  = 237.24 cm) of *P. glauca* were mature.

In the deep sea habitats, 37 species from 11 families were represented (Table I). The family Squalidae was dominant, contributing with 19 species to over 90% of all chondrichthyans captured. Dominant genera were *Squalus*, *Centrophorus*, *Etmopterus* and *Galeus*. Taiwan gulper shark, *Centrophorus niaukang*, was reported from the Ryukyu Is. (Yano and Kugai, 1993a), and other five species have occurred in the waters around the Ryukyu Is. The rare species, *Somniosus longus*, was collected from off the Okinawa Is. at the depth of 1116 m.

#### *Parnaturus melanobranchius*

Seven specimens of the blackgill catshark, *Parnaturus melanobranchius*, were collected from the deep sea habitats of the Ryukyu Is. (Fig. 3). Part of the specimens were deposited in the museums (CBM 5149-5152). This species was known to be distributed in the South China Sea (Chen, 1966; Compagno, 1984). This is the first record of this species from the waters around Japan.

Proportional dimensions in percentage of TL of *P. melanobranchius* are shown in table II. In addition, measurements of the related species of the genus including type specimens are also given for comparison (Table III). *Parnaturus pilosus* was measured from Ryukyu Is. (KMNH VR01308, 01314, 01331, 01355, 01372 and 01376) and Suruga Bay (TMFE, 2016, 34°21'N - 138°24'E, 358-750 m in depth) specimens. Proportional dimensions in percentage of total length of the Ryukyu Is. specimens are slightly different from the measurements of the type specimen of *P. melanobranchius* (Tables II, III;

Table II. - Proportional dimensions, in percentage of TL, of *Parmaturus melanobranchius* collected from the Ryukyu Is. (5 males, 639-781 mm TL and 2 females, 692-730 mm TL) and of *P. pilosus* collected from the Ryukyu Is. (5 females, 502-638 TL) and Suruga Bay (453 mm TL). SD: standard deviation.

	<i>Parmaturus melanobranchius</i>				<i>Parmaturus pilosus</i>			
	Range		Mean	SD	Range		Mean	SD
<b>Total length</b>	639	781	697.86	51.976	453	638	533.67	62.260
<b>Snout tip to:</b>								
outer nostrils	2.60	4.24	3.41	0.602	2.00	3.28	2.76	0.450
eye	4.93	6.80	5.94	0.579	4.36	5.74	5.11	0.576
spiracle	10.14	12.04	10.86	0.727	9.85	11.15	10.43	0.538
mouth	4.93	6.49	5.41	0.542	4.86	5.30	5.16	0.170
1st gill opening	13.84	16.62	15.94	0.978	14.13	15.69	15.13	0.662
3rd gill opening	14.52	18.55	17.55	1.382	16.11	18.43	17.12	0.761
5th gill opening	17.26	20.11	19.33	0.979	17.88	20.55	19.67	0.984
pectoral origin	16.99	20.40	18.80	1.014	17.22	20.07	19.23	1.050
pelvic origin	46.68	49.77	48.22	1.093	44.15	47.65	46.21	1.450
cloaca	49.42	52.74	51.36	1.170	47.02	50.47	49.17	1.309
1st dorsal origin	52.48	54.15	53.29	0.521	44.37	48.73	46.94	1.561
2nd dorsal origin	67.82	69.45	68.59	0.644	63.13	65.83	64.66	0.942
anal fin origin	59.97	61.64	61.05	0.582	54.30	58.18	56.90	1.615
upper caudal origin	78.50	80.20	79.49	0.592	69.76	73.27	71.63	1.376
lower caudal origin	72.33	80.78	74.22	2.936	68.65	72.26	70.71	1.343
<b>Distance between bases:</b>								
1st and 2nd dorsal	8.21	9.89	9.34	0.559	10.04	12.35	11.38	1.025
2nd dorsal and caudal	1.80	3.09	2.39	0.494	1.20	2.01	1.65	0.299
pectoral and pelvic	23.41	29.45	25.91	2.184	14.05	24.91	21.93	3.978
pelvic and anal	7.04	8.50	7.92	0.505	3.64	6.62	4.63	1.067
anal and caudal	0.55	1.79	1.14	0.417	0.94	1.64	1.39	0.267
<b>Distance between insertions of :</b>								
pectoral and pelvic	27.51	33.56	30.18	2.062	26.93	29.47	28.28	1.002
<b>Nostrils:</b>								
distance between inner corners	2.05	2.47	2.29	0.147	1.10	2.92	2.38	0.655
<b>Mouth:</b>								
width	9.27	10.96	10.02	0.542	8.83	11.55	10.18	0.964
length	3.01	4.06	3.61	0.473	3.62	3.84	3.75	0.322
<b>Labial furrow lengths:</b>								
upper	1.45	1.95	1.68	0.210	0.98	1.20	1.10	0.120
lower	1.64	1.95	1.82	0.134	1.85	2.00	1.99	0.113
<b>Gill opening lengths:</b>								
1st	1.08	1.92	1.54	0.278	1.57	2.54	1.99	0.388
3rd	1.37	2.33	1.73	0.354	1.32	2.73	2.14	0.487
5th	0.68	1.55	1.12	0.280	0.66	1.45	0.96	0.269
<b>Spiracle:</b>	0.31	0.68	0.50	0.154	0.39	0.91	0.69	0.194
<b>Eye:</b>								
horizontal diameter	4.23	5.20	4.76	0.310	4.64	7.48	5.30	1.088
vertical diameter	0.90	1.24	1.03	0.115	0.91	1.82	1.41	0.323

Table II. - (continued)

	<i>Parmaturus melanobranchius</i>				<i>Parmaturus pilosus</i>			
	Range		Mean	SD	Range		Mean	SD
<b>Interorbital width:</b>	6.03	7.11	6.53	0.386	5.96	7.28	6.64	0.545
<b>1st dorsal fin:</b>								
overall length	6.71	7.94	7.33	0.536	7.85	10.38	9.29	0.996
length base	5.10	7.11	5.92	0.784	6.18	6.91	6.36	0.278
length post. margin	1.72	3.42	2.85	0.647	4.39	5.47	4.78	0.425
height	3.46	3.91	3.63	0.176	5.11	5.82	5.38	0.270
<b>2nd dorsal fin:</b>								
overall length	7.73	11.56	10.03	1.174	8.39	9.27	8.86	0.320
length base	6.65	10.55	8.84	1.213	5.18	6.39	5.80	0.420
length post. margin	4.10	5.34	4.64	0.373	3.82	5.08	4.12	0.480
height	4.38	5.62	5.03	0.475	4.64	5.84	5.23	0.490
<b>Anal fin:</b>								
overall length	11.56	12.59	12.17	0.418	13.48	14.79	13.93	0.469
length base	10.40	12.04	11.36	0.612	12.18	13.91	12.69	0.645
length post. margin	4.79	7.53	6.35	0.886	7.48	8.83	8.08	0.437
height	2.46	4.64	3.77	0.663	4.56	5.58	5.11	0.399
<b>Pectoral fin:</b>								
length base	4.64	5.63	5.04	0.320	5.52	6.36	5.97	0.308
length ant. margin	7.11	9.27	8.40	0.687	8.61	10.00	9.27	0.505
length distal margin	2.17	4.11	3.17	0.776	4.20	5.49	4.75	0.464
length post. margin	4.79	7.23	6.59	0.821	6.84	8.96	8.17	0.755
<b>Pelvic fin:</b>								
overall length	8.61	10.12	9.24	0.553	9.05	10.66	9.88	0.644
length base	5.87	7.95	6.56	0.828	6.62	8.93	7.55	0.810
length ant. margin	5.88	7.23	6.46	0.560	5.08	7.44	6.20	1.008
length distal margin	1.20	2.88	1.89	0.597	2.19	3.52	2.88	0.516
length clasper	7.82	9.27	8.49	0.522	-	-	-	-
length clasper (outer)	4.17	5.01	4.65	0.310	-	-	-	-
<b>Caudal fin:</b>								
length dorsal lobe	19.32	20.95	20.27	0.678	25.86	29.36	27.48	1.386
length ventral lobe	12.83	16.42	14.90	1.261	9.93	11.86	11.27	0.743
dorsal tip to notch	3.83	4.66	4.28	0.284	4.18	5.98	4.83	0.607
depth notch	3.15	3.83	3.42	0.245	3.64	4.86	4.47	0.454
<b>Trunk at pectoral origin:</b>								
width	10.82	13.56	12.68	0.959	11.04	13.64	12.32	0.897
height	7.82	11.10	9.08	1.101	8.96	11.91	10.52	1.344

holotype, BMNH1965.8.11.6, 20°05'N - 115°03'E, 546 m in depth). However, I think that the type specimen is a small immature specimen and differences between the holotype and the Ryukyu Is. specimens are only ontogenetic variations. In both specimens, the origin of the first dorsal fin is more posteriorly located than the origin of the pelvic fins.

Table III. - Proportional dimensions, in percentage of TL, of the type specimens of *Parmaturus melanobranchius*, *P. pilosus*, *P. campechiensis* and *P. xaniurus*.

	<i>Parmaturus melanobranchius</i> BMNH 1965.8.11.6 Holotype Female	<i>Parmaturus pilosus</i> MCZ 1107S Holotype Male	<i>Parmaturus campechiensis</i> USNM 206184 Holotype Female	<i>Parmaturus xaniurus</i> USNM 46719 Lectotype Female
<b>Total length (mm):</b>	220	435	155	545
<b>Snout tip to:</b>				
outer nostrils	3.18	2.30	3.23	2.39
eye	7.73	4.14	7.10	5.14
spiracle	12.73	10.11	12.26	9.91
mouth	6.82	4.83	5.81	4.04
1st gill opening	17.27	14.94	16.13	17.25
3rd gill opening	18.64	17.01	17.42	21.65
5th gill opening	21.36	18.85	19.35	26.06
pectoral origin	20.45	18.39	18.71	24.22
pelvic origin	38.18	41.84	39.35	45.14
cloaca	39.09	46.21	51.61	48.99
1st dorsal origin	45.00	43.91	39.35	46.97
2nd dorsal origin	58.64	61.61	54.84	65.32
anal fin origin	50.91	54.25	51.61	61.47
upper caudal origin	72.27	68.97	64.52	75.96
lower caudal origin	65.45	69.20	60.65	75.41
<b>Distance between bases:</b>				
1st and 2nd dorsal	10.91	10.34	9.68	11.56
2nd dorsal and caudal	2.73	1.38	1.94	2.57
pectoral and pelvic	15.00	18.39	16.77	17.43
pelvic and anal	5.91	5.98	3.23	7.89
anal and caudal	4.55	2.07	0.65	2.57
<b>Distance between insertions of:</b>				
pectoral and pelvic	20.45	23.22	20.65	21.83
<b>Nostrils:</b>				
distance between inner corners	2.27	2.07	3.23	1.65
<b>Mouth:</b>				
width	8.18	8.74	9.03	10.64
length	3.18	3.22	1.94	4.59
<b>Gill opening lengths:</b>				
1st	1.36	1.84	2.58	2.02
3rd	0.91	1.15	1.29	2.75
5th	0.91	0.69	0.65	2.02
<b>Spiracle:</b>	0.45	0.69	0.65	0.55
<b>Eye:</b>				
horizontal diameter	3.18	4.83	4.52	4.40
vertical diameter	1.36	0.69	1.29	0.92
<b>Interorbital width:</b>	7.27	7.36	6.45	7.71



Table III. - (continued).

	<i>Parmaturus melanobranchius</i> BMNH 1965.8.11.6 Holotype Female	<i>Parmaturus pilosus</i> MCZ 11075 Holotype Male	<i>Parmaturus campechiensis</i> USNM 206184 Holotype Female	<i>Parmaturus xaniurus</i> USNM 46719 Lectotype Female
<b>1st dorsal fin:</b>				
overall length	9.09	11.26	10.97	9.17
length base	5.91	8.05	6.45	6.06
length post. margin	2.27	3.91	3.87	3.49
height	2.73	4.37	3.23	3.67
<b>2nd dorsal fin:</b>				
overall length	11.36	10.11	12.26	10.09
length base	10.00	6.90	8.39	6.79
length post. margin	3.18	4.14	3.23	3.30
height	3.64	4.37	3.23	3.67
<b>Anal fin:</b>				
overall length	14.14	14.71	12.26	11.74
length base	12.87	12.64	10.32	9.91
length post. margin	6.22	8.74	3.87	6.61
height	4.61	5.06	3.87	4.59
<b>Pectoral fin:</b>				
length base	4.55	5.52	5.81	4.40
length ant. margin	9.55	9.20	9.03	11.19
length distal margin	5.91	6.44	5.81	7.71
length post. margin	6.36	7.59	5.16	6.97
<b>Pelvic fin:</b>				
overall length	11.36	10.57	7.74	11.56
length base	5.91	7.13	5.81	8.26
length ant. margin	4.55	7.13	3.23	5.87
length distal margin	2.73	2.07	1.29	4.40
length clasper	-	5.06	-	-
length clasper (outer)	-	2.30	-	-
<b>Caudal fin:</b>				
length dorsal lobe	25.45	29.43	33.55	25.50
length ventral lobe	12.27	11.49	12.90	8.99
dorsal tip to notch	2.73	4.37	3.23	3.49
depth notch	4.55	5.06	6.45	4.59
<b>Trunk at pectoral origin:</b>				
width	11.36	10.80	12.90	13.03
height	8.18	10.80	9.03	7.34

This character clearly distinguishes *P. melanobranchius* from the other species of the genus. As a result, the specimens of the Ryukyu Is. are identified to *P. melanobranchius*. This is the second species of *Parmaturus* recorded from Japan.

Number of turns in the spiral valve ranged from 10-11 ( $\bar{x} = 10.14$ ,  $n = 7$ ) for *P. melanobranchius* and from 7-9 ( $\bar{x} = 7.80$ ,  $n = 5$ ) for *P. pilosus*. Total, monospondylous, caudal and precaudal vertebral numbers for *P. melanobranchius* ranged from 149-151 ( $\bar{x} = 150.00$ ,  $n = 3$ ), 46-47 ( $\bar{x} = 46.33$ ), 57-61 ( $\bar{x} = 59.33$ ), and 89-93 ( $\bar{x} = 90.67$ ), respectively. Total, monospondylous, caudal and precaudal vertebral numbers for *P. pilosus* ranged from 131-142 ( $\bar{x} = 136.80$ ,  $n = 5$ ), 40-43 ( $\bar{x} = 41.60$ ), 59-65 ( $\bar{x} = 62.8$ ), and 72-78 ( $\bar{x} = 74.00$ ), respectively.

*Parmaturus melanobranchius* differs from the Japanese species, *P. pilosus* (holotype, MCZ 1107S, 34°59'N-139°31'E, Garman, 1906), in having a more posteriorly located dorsal fins (Fig. 3), large numbers of spiral valves and vertebrae, and larger maturity sizes. *P. melanobranchius* is also distinguishable from *P. campechiensis* from Gulf of Mexico (Springer, 1979; holotype, USNM 206184, 21°33'N-96°48'W), *P. macmillani* from New Zealand (Hardy, 1985), and *P. xaniurus* from Central California to Gulf of California, Mexico (Gilbert, 1892, lectotype, USNM 46719, 33°55'N-120°28'W) by having the more posteriorly located first dorsal fin (Table III). Last and Stevens (1994) reported *Parmaturus* sp. A from the Saumarez Plateau off northeastern Australia. This species has more posteriorly located dorsal fins as in *P. melanobranchius*. I think that the present specimens are *P. melanobranchius*, not the Last and Stevens' *Parmaturus* sp. A, because the localities of the present specimens are close to the type locality of *P. melanobranchius*.

The blackgill catshark, *P. melanobranchius*, was described by Chen (1966) from the South China Sea. Chu *et al.* (1983) described a new species, *Figaro piceus*, from the South China Sea. Compagno (1984) stated that *F. piceus* is a synonym of *P. melanobranchius*. Nakaya *et al.* (pers. com.) suggested that *P. melanobranchius* (their tentative Japanese name is Koshinagaimori-zame) was collected from the Okinawa (abstract of the Annual Meeting of the Ichthyological Society of Japan, 1985). I collected specimens of *Parmaturus* sp. during the 1984 surveys of the Okinawa Prefectural Fisheries Experiment Station around the Ryukyu Is., and it was identified as *P. melanobranchius*. Yano and Kugai (1993b) reported that two species, *P. pilosus* and *Parmaturus* sp. occur in the waters around the Ryukyu Is. The specimens of *Parmaturus* sp. of Yano and Kugai (1993b) are *P. melanobranchius*. However, no other material exists from the waters around Japan. In the present report, at least, *P. melanobranchius* and *P. pilosus* are both distributed around the Ryukyu Is., Japan.

*Parmaturus melanobranchius* was captured from off of the Hateruma Is. (24°09'N-123°58'E and 24°05'N-123°18'E), of the Yonaguni Is. (24°03'N-122°59'E and 24°29'N-123°06'E), and of the Minna Is. (24°50'N-124°26'E) at the depth from 540 m to 835 m. Around the Ryukyu Is., *P. pilosus* was collected from off the Ishigaki Is. (24°43'N-124°26'E and 24°09'N-124°10'E) and the Yonaguni Is. (24°29'N-122°56'E and 24°11'N-122°38'E) at the depth from 520 m to 895 m.

**Acknowledgements.** - I thank K. Kugai, H. Fujita, O. Abe, H. Shimizu, T. Kosuge, staff members of Okinawa Prefectural Fisheries Experiment Station and Seikai National Fisheries Research Institute, Ishigaki Toropical Station, captain and crew members of the research vessels "Tonan Maru", "Shonan Maru", "Wakatori Maru" and "Yaeyama", and fishermen of Yaeyama Is. for their valuable cooperation of sampling during the surveys.

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## Symposium 7

### ENDANGERED SPECIES, CONSEQUENCES OF SPECIES INTRODUCTION

Chairperson: **Christian LÉVÊQUE**

- BRUCE B.D., GREEN M.A. & P.R. LAST. - Aspects of the biology of the endangered spotted handfish, *Brachionichthys hirsutus* (Lophiiformes: Brachionichthyidae) off Southern Australia. 369-380  
*Éléments de la biologie du poisson-crapaud tacheté, Brachionichthys hirsutus (Lophiiformes: Brachionichthyidae), espèce en danger des côtes méridionales d'Australie.*
- CAPULI E. & R. FROESE. - Status of the freshwater fishes of the Philippines. 381-384  
*Statut des poissons d'eau douce des Philippines.*
- CASAL C.M.V., BARTLEY D.M., FROESE R., SA-A P. & M.L.D. PALOMARES. - Documenting the status of freshwater fish introductions in Oceania. 385-392  
*Bilan des introductions de poissons d'eau douce en Océanie.*
- GILL H.S., HAMBLETON S.J. & D.L. MORGAN. - Is the mosquitofish, *Gambusia holbrooki* (Poeciliidae), a major threat to the native freshwater fishes of South-western Australia ? 393-403  
*Gambusia holbrooki (Poeciliidae) est-elle une menace pour les poissons indigènes des eaux douces du sud-ouest de l'Australie ?*



## ASPECTS OF THE BIOLOGY OF THE ENDANGERED SPOTTED HANDFISH, *BRACHIONICHTHYS HIRSUTUS* (LOPHIIFORMES: BRACHIONICHTHYIDAE) OFF SOUTHERN AUSTRALIA

by

Barry D. BRUCE, Mark A. GREEN & Peter R. LAST (1)

**ABSTRACT.** - The Brachionichthyidae (handfishes) is a little known lophiiform family endemic to SE Australia. Some of the inshore species have extremely restricted distributions, a feature that makes their populations highly vulnerable to disturbance. The spotted handfish (*Brachionichthys hirsutus*) is endemic to a small area of SE Tasmania and was common throughout the lower Derwent estuary and adjoining bays prior to the mid 1980's. It has since suffered a serious decline in distribution and abundance. In June 1996, a study of *B. hirsutus* commenced to establish a biological profile for developing appropriate conservation strategies for the species. *B. hirsutus* is a small, benthic, slow moving, species that is easily approached and measured underwater. Individuals may be identified by their unique markings, which enables individual growth trajectories and movement patterns to be mapped. Growth rates of female *B. hirsutus* suggest that maturity is reached after 2-3 years at a size of 75-80 mm. Small numbers (80-250) of large eggs are deposited in an interconnected egg mass that is structurally unique amongst antennarioids and is wrapped around a vertical object on the bottom. The female guards the eggs which take 7-8 weeks to hatch. Handfish lack a dispersive larval stage and hatch as well-formed juveniles (6-7 mm in length) that settle immediately to the bottom. Consequently, the ability for handfish to repopulate areas from which they have been removed is expected to be low. The cause of the decline in *B. hirsutus* has yet to be determined. Suggested reasons include predation or habitat disturbance by a recently introduced asteroid and/or habitat modification through rural, industrial and urban development..

**RÉSUMÉ.** - Éléments de la biologie du poisson-crapaud tacheté, *Brachionichthys hirsutus* (Lophiiformes: Brachionichthyidae), espèce en danger des côtes méridionales d'Australie.

Les Brachionichthyidae constituent une petite famille de poissons-crapauds endémiques des côtes sud-est de l'Australie. Certaines espèces littorales ont une distribution extrêmement réduite et sont très vulnérables aux perturbations de leur milieu. Ainsi, le poisson-crapaud tacheté, *Brachionichthys hirsutus*, a une distribution limitée à une petite zone de la côte sud-est de la Tasmanie, alors qu'il était commun dans toute la partie inférieure de l'estuaire de la rivière Derwent et dans les baies adjacentes avant le milieu des années 1980. Depuis, cette espèce a subi un déclin prononcé de sa distribution et de son abondance. En juin 1996, sa biologie a été étudiée pour déterminer la meilleure stratégie de conservation. *B. hirsutus* est une petite espèce benthique, indolente, que les plongeurs peuvent approcher facilement pour l'observer. Les individus peuvent être identifiés par leur patron de coloration, aussi est-il possible de suivre leur croissance et de cartographier leurs mouvements. Les taux de croissance des femelles suggèrent que la maturité est atteinte au bout de 2 à 3 ans pour une taille variant de 75 à 80 mm LT. Un petit nombre (80-250) de gros oeufs est déposé en une masse unique de structure particulière, qui est enroulée autour d'un support vertical sur le fond. Les femelles gardent les oeufs qui éclosent au bout de 7 à 8 semaines. Il n'y a pas de phase larvaire de dispersion; à l'éclosion, les jeunes poissons-crapauds tachetés ont 6-7 mm LT et s'installent immédiatement sur le fond. De ce fait, la capacité de ces poissons-crapauds à repeupler les zones où ils ont été décimés est très faible. Mais la véritable raison de leur déclin n'est pas encore clairement établie. Parmi les causes possibles, on peut évoquer la

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prédation et la modification de leur habitat par l'invasion récente d'étoiles de mer et/ou par le développement rural, industriel et urbain.

Key-words. - Brachionichthyidae, *Brachionichthys hirsutus*, PSE, Southern Australia, Tasmania, Handfish, Reproduction, Endangered species, Conservation.

The lophiiform family Brachionichthyidae (handfishes) is the most speciose of marine fish families that are endemic to Australia. Some representatives of the family have among the narrowest ranges of any of the 4300, or so, marine fish known from the region (Last *et al.*, 1983; Yearsley *et al.*, 1997). Five of the eight currently identified species are endemic to Tasmania and Bass Strait (Last *et al.*, 1983). The red handfish (*Sympterichthys politus*) and an undescribed species (Ziebell's handfish, *Sympterichthys* sp.) appear to be confined to a few restricted, shallow reef habitats in south-eastern Tasmania. The spotted handfish (*Brachionichthys hirsutus*) is endemic to the lower Derwent River estuary and adjoining bays and channels (Edgar *et al.*, 1982; Last *et al.*, 1983).

The small population sizes and highly restricted distributions of all the inshore endemic Tasmanian handfishes make them vulnerable to disturbance. However, only the spotted handfish is known to have suffered a serious, recent, population decline (Barrett *et al.*, 1996). The species has been listing as « endangered » and « critically endangered » under the Australian Endangered Species Act and IUCN Red List respectively, but the cause of the decline is unknown. Suggested reasons have included predation on egg masses or disturbance of benthic communities by the recently introduced northern Pacific seastar, *Asterias amurensis*, habitat modification through increased siltation, heavy metal contamination and urban effluent (Last and Bruce, 1997; Bruce *et al.*, 1998).

The biology of brachionichthyids is poorly documented, apart from aspects of their morphology, osteology and distribution (see Pietsch, 1981; Edgar *et al.*, 1982; Last *et al.*, 1983; Gomon *et al.*, 1994). They are small, colourful, slow moving benthic fishes that are easily approached and photographed. Last *et al.* (1983) reported that *B. hirsutus* attached its eggs to solid objects on the bottom via thin threads and that its diet consisted of small shellfish, shrimps and polychaete worms. Whitley (1949) described an adult female *B. hirsutus* with eggs extruding from the body and a 14 mm juvenile *Sympterichthys verrucosus*. The lack of information about the biology, population dynamics and habitat requirements has proved the major impediment to identifying the cause of the decline in the size of the population of *B. hirsutus*, in further defining the conservation status of the group in general and in establishing appropriate conservation strategies.

In June 1996 we commenced a study of *Brachionichthys hirsutus* in order to establish a biological profile for developing appropriate conservation strategies for the species. The study involved concurrent field and laboratory components, the latter to further detail both biological and behavioural aspects, as well as establishing captive husbandry techniques. This paper describes our field observations on age, growth and reproduction of a population within the lower Derwent Estuary over the period June 1996 - November 1997.



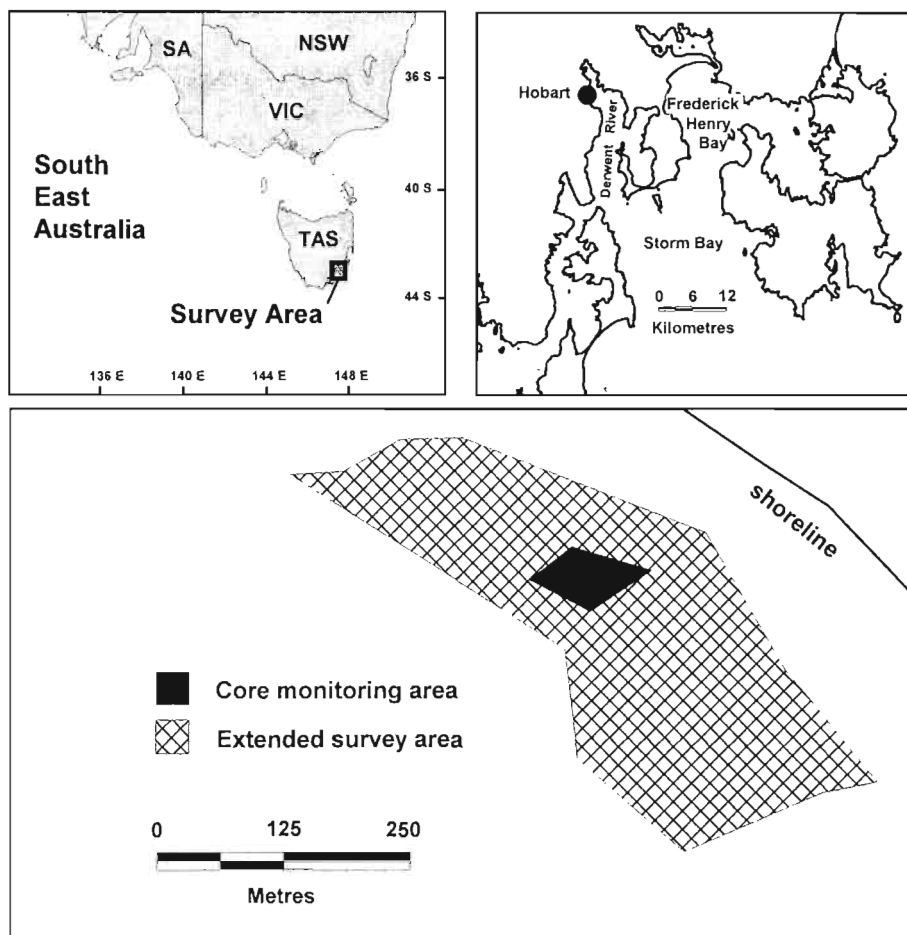


Fig. 1. - The study area in south eastern Tasmania which approximates the previous known range of *Brachionichthys hirsutus*. The survey area in the lower reaches of the Derwent River estuary is not identified to ensure the security of the population.

## MATERIAL AND METHODS

### Study area

The study site was located in a shallow bay of the lower Derwent estuary near Hobart Tasmania (Fig. 1). The precise location of the site has not been shown to maintain the security of the population. The site was selected on the basis of surveys in 1996 and early 1997 covering the previous known distribution of the species (Barrett *et al.*, 1996; Bruce *et al.*, 1997). The core survey area was approximately 70 m x 70 m, bounded by the 6 and 8 m depth contours. The substrate was primarily medium to coarse sand and silt with occasional, isolated, low relief rocks and numerous shallow shell-lined depressions.



Fig. 2. - *Brachionichthys hirsutus*, 64 mm TL.

This area was monitored approximately monthly between June and August 1996 and between January and October 1997. The area was monitored more frequently (approximately fortnightly) during the 1996 spawning and hatching period (September - December). Additional surveys were conducted on an extended area of the bay (500 m x approximately 120 m, centred on the core area and bounded by the 5 and 10 m depth contours) in 1997 to provide an index of population size, population fecundity, monitor recruitment and record extended movement patterns. Results reported herein refer primarily to field observations within the core survey area, although data on growth includes that collected on the extended area surveys and some observations on reproductive behaviour and early life history are included from laboratory rearing. Data on patterns of movement, population indices, population dynamics and recruitment will be covered in a subsequent publication.

#### Monitoring methods

The site was searched via SCUBA for handfish during each dive. The location of individuals was initially marked, for future reference, by numbered steel stakes, 40 cm in length, that were driven approximately 10 cm into the substrate. In subsequent dives, the position of located individuals was plotted based on their distance and direction from the nearest stake. If an existing stake could not be found, a new stake was positioned adjacent to the fish.

For each fish, total length (measured with vernier callipers), adjacent substrate type and proximity to any bottom features (e.g., shell-lined depressions, low relief rocks)

were recorded. A search of the surrounding area for egg masses was also made. Total length was recorded in preference to standard length as the latter was extremely difficult to measure *in situ*.

The hypothesized role of *Asterias amurensis* in the decline of *B. hirsutus* prompted us to monitor densities of the seastar in the sample area. Densities were initially estimated from two 50 m transects (2 m width) and then from four tracks of 100 m length in 1997.

### Identification of individuals

*B. hirsutus* have individually recognisable patterns of spots that do not change with season, substrate type or behaviour (Fig. 2). As a reference for their identification, the left side of each fish observed was photographed, *in situ*. Some changes in pattern occur with growth (e.g., a streak may break into two or more spots), however these changes are easily traced. The ability to easily measure specimens *in situ*, and identify individual fish allowed us to verify growth rate data and plot movement patterns.

## RESULTS

A total of 130 individuals of *Brachionichthys hirsutus* (excluding newly hatched juveniles < 10.0 mm) were photographed and registered during the study period in the combined core and extended survey areas. Ninety-five individuals were recorded within the core study area. This reflected the higher survey effort in the core area compared to that in the extended survey area.

### Resightings

Twenty-eight fish (21% of the total observed) were resighted during the study period, most resightings occurring in the core area. The time intervals between successive resightings ranged from 2 to 329 days. The most frequently resighted fish (excluding those monitored with eggs) was recorded on 5 separate dates spanning a 145 day period between July and December 1996.

### Reproduction

Four egg masses were observed in the core area during 1996, on 30 September, 15 October (two masses) and 5 November. No egg masses were observed in the core area in 1997, although two egg masses were recorded in the extended survey area on 8 and 9 October.

In each case, egg masses of 60-100 eggs were wrapped around the base of a stalked ascidian, *Sycozoa* sp. Each egg was separately housed in an elliptical, membranous, flask-shaped structure, the neck of which had a prominent cup-shaped central pore, as well as a thin tendril and a larger diameter tubule on opposing sides (Fig. 3). The surface of each flask was covered with short, papillose, projections, each approximately 0.2 mm in length. Tendrils, which were individual non-branching structures that formed a tangled web within and around the egg mass, probably assisted in anchoring it to the substrate. Tubules, however, formed a continuous branching system that linked each flask and held the egg mass together. Both tendrils and tubules appeared to be hollow. Flasks and eggs with full term embryos were approximately 5.5-6.0 mm length (4.2-4.3 mm width) and 3.9-4.2 mm length (2.8-3.0 mm width) respectively.

Three of the egg masses located in 1996 were revisited, and the development of two of them was followed in detail. The same adult was present immediately adjacent to its

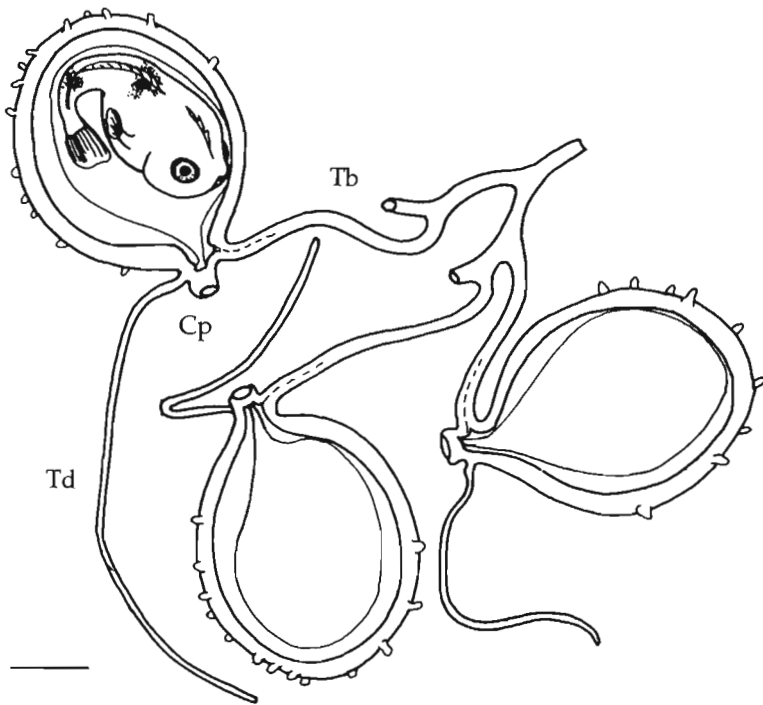


Fig. 3. - Structure of *Brachionichthys hirsutus* egg mass. Cp: central pore; Td: tendril; Tb: interconnecting branched tubule. Scale bar = 2 mm.

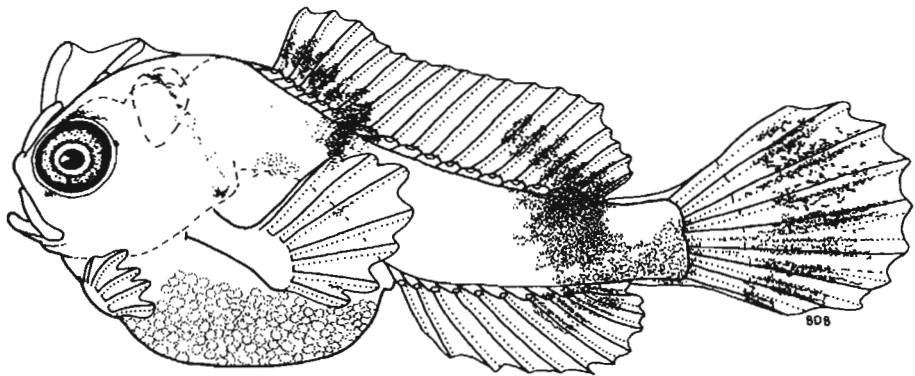


Fig. 4. - Newly hatched *Brachionichthys hirsutus*, 5.2 mm SL, 6.8 mm TL.

respective egg mass on each occasion visited. One of the adults, specimen B23, was observed with a notably distended abdomen 32 days prior to being sighted next to an egg mass. Based on these field observations and the spawning and subsequent guarding behaviour of three captive females, we conclude that only the female guards the egg mass.

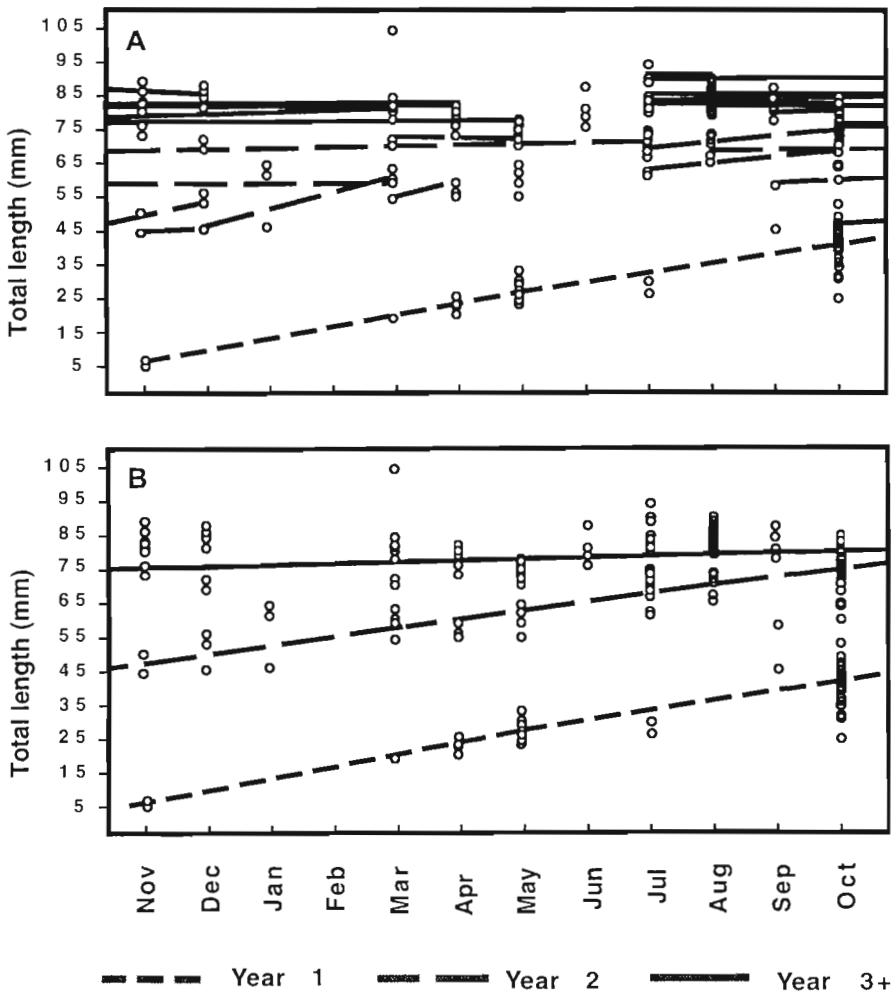


Fig. 5. - Growth rate of *Brachionichthys hirsutus* within Derwent estuary. A: Growth of resighted fish. Lines denote growth records for resighted fish. (B): Population growth rates inferred from monthly length data.

Specimen B23 was first observed with eggs on 30 September. Eggs were relatively clean, with little epiphytic growth. The external surface of the eggs gradually became discoloured with algae and detritus during the period of development. Embryos were first noticed on 28 October, at which time eyes were well-developed and pectoral fins were visible. The egg mass was still intact on 5 November with no sign of hatching. Poor visibility and bad weather prevented us from revisiting the site until 18 November, at which time hatching was almost complete with only three embryos still in eggs. A search of the surrounding area located 6 juveniles (7.9-9.1 mm in length) at distances ranging between 1.5-3.2 m from the remains of the egg mass. Based on growth rates observed in the laboratory, we estimate these individuals to have hatched 5-7 days earlier. The female

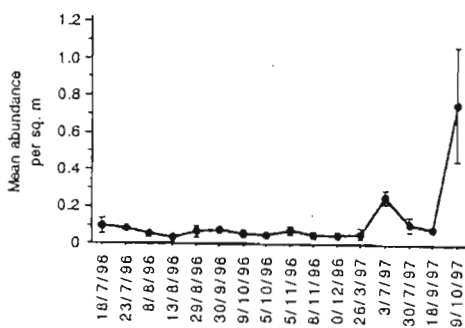


Fig. 6. - Mean abundance of *Asterias amurensis* per m<sup>2</sup> within the core survey area, July 1996 - October 1997. Bars denote 1 standard deviation.

was also observed away from the egg mass for the first time since spawning and was located at a distance of 3 m.

Specimen B39 was first observed adjacent to an egg mass on 15 October, with eggs that were noticeably discoloured on the external surfaces and no embryos visible. Embryos were visible on the subsequent visit (28 October). On 5 November, embryos were large and well developed. A search of the surrounding substrate located a single 7 mm juvenile. Hatching of this egg mass had completed by 18 November. Only a single juvenile was observed on that date, 5 m from the remains of the egg mass. The adult, B39, was not resighted.

### Juveniles

In the laboratory, *B. hirsutus* hatched at 6.7-6.9 mm TL (5.2-5.5 mm SL). Newly hatched fish were well developed with full fin complements (including the illicium), functional eyes and functional mouth (Fig. 4). A large, internal, yolk reserve was present at hatching and diminished over the following few days. Newly hatched juveniles were predominantly white. Melanophores were present over the anterior-most and posterior-most 7-8 rays of the second dorsal fin, in both cases extending laterally down the body surface to form two broad bands. The posterior band extended onto the caudal peduncle and ventrally over the posterior-most 5-6 rays of the anal fin. Melanophores were also present in a broad band over the distal margin of the caudal fin and scattered over the head (primarily around the orbit). Some melanophores were present internally around the otic capsule and above the gut.

### Age and growth

Based on the recorded lengths during surveys, *B. hirsutus* grew at a relatively constant rate between November and May, increasing in size from 6-7 mm at hatching to 20-30 mm (Fig. 6). By October-November, this mode could be traced to a size of 35-50 mm which we assume to represent the size at age 1. This mode was subsequently traced to approximately 55-65 mm by May and then links to 65-80 mm in the October - November series, presumably representing size at year 2. Growth rates between age 1 and age 2 (1 + fish) were corroborated by the lengths of resighted juveniles (Fig. 5a).

Growth rates appeared to slow dramatically after approximately 70–80 mm and it was not possible to discern year classes after this point from length data. Again this was corroborated by length measurements on resighted individuals (Fig. 5a).

### *Asterias amurensis*

The density of *A. amurensis* remained relatively stable between July 1996 and July 1997 at approximately 1 per 10 m<sup>2</sup>. A significant increase in abundance was recorded during October 1997 reaching a peak value of 8 per 10 m<sup>2</sup> (Fig. 6).

## DISCUSSION

The ability to identify individual *Brachionichthys hirsutus* based on their unique pigment patterns provided an ideal opportunity to examine growth, movement and aspects of reproductive dynamics that would otherwise be extremely difficult (or destructive). Unlike the changes observed in colour patterns of antennariids (Pietsch and Grobecker, 1987), we found no evidence that pigment patterns vary diurnally or in response to substrate type or behaviour. All captive individuals maintained their unique patterns and resighted individuals were readily identified based on photographic records up to 329 days after first being sighted.

### Reproduction

*B. hirsutus* has a complex egg mass that is unique among the antennarioids. Tendrils and interconnecting tubules between egg flasks assist in attaching the egg mass to the substrate and maintaining the integrity of the egg mass during the incubation period. Whether tubules serve any other function after spawning or if they are just remnant structures derived from the ovary is unknown. The large, cup-shaped central pore located at the top of the egg flask may provide an entry point for sperm during fertilisation and the flask itself may protect eggs from predation and abrasion. As we have observed similar egg masses for both the red handfish (*Sympterychthys politus*) and Ziebell's handfish (*Sympterychthys* sp.), it is likely that such egg mass structures are characteristic of brachionichthyids.

Large, benthic eggs, low fecundity and parental care are unusual in lophiiform fishes and have only been reported in a few species of antennarioids (Pietsch and Grobecker, 1987; Kuiter, 1993; Pietsch, 1997, pers. comm.). Of these species, 5 are known to either carry eggs attached to the body (i.e., *Lophiocharon trisignatus*, *L. lithinostomus*, *Tetrabrachium ocellatum*) or as a cluster held in a pocket formed by folding the pectoral and caudal fins against the body (i.e., *Histiophryne cryptacanthus*, *H. bogainvilli*) (Pietsch and Grobecker, 1987). Only two species (i.e., *Rhycherus filamentosus* and *Echinophryne crassispina*) are reported to attach eggs to the substrate (Kuiter, 1993). In all cases, eggs were reported to be attached to each other, usually by single or double-stranded acellular filaments or within mucoid sheaths. Eggs attached to common branched filaments have only been reported for *Tetrabrachium ocellatum* (Pietsch and Grobecker, 1987).

Egg masses of *B. hirsutus* were only located around the base of the stalked ascidian *Sycozoa* sp. in the survey area, although egg masses have recently been observed around other vertical objects at a site outside the Derwent estuary (J. Ross, Univ. Tasmany, 1997, pers. comm.). Individuals will spawn around rigid vertical structures in aquaria. Similarly,

we have only observed egg masses of *S. politus* around vertical structures (*Caulerpa simpliciuscula*), while eggs of *Sympterichthys* sp. have been observed wrapped around a vertical sponge (K. Gowlett-Holmes, 1996, pers. comm.). The similarity between species of both genera in egg mass structure and the orientation of spawning substrate suggests that brachionichthyids prefer to attach their egg masses to isolated vertical structures during spawning.

#### Age and growth

This study provides the first preliminary information on age and growth for an antennarioid, apart from observations on developmental rates of larval and early juveniles stages of *Histrio histrio* (see Dooley, 1972; Martin and Drewry, 1978; and references within). Based on observed length frequencies, resighted individuals and the minimum size of females observed with egg masses, maturity in female *B. hirsutus* is attained within the Derwent population after 2-3 years of age, at a size of about 75-80 mm. The growth rate appears to slow dramatically thereafter to only a few millimetres per year. Size at maturity for males is yet to be established as we are unable to differentiate between sexes using external characters. However, the smallest male to fertilize eggs in our captive rearing program was 87 mm TL. Although Barrett *et al.* (1996) reported a 117 mm specimen from the Derwent and we sighted a 105 mm individual during our surveys, longevity is yet to be determined.

#### Causes of decline

Both the cause of the decline in *B. hirsutus* and the period over which it has occurred is unclear. Anecdotal records suggest a relatively rapid decline in abundance occurred in the mid to late 1980's, at least in the vicinity of Hobart. Bruce *et al.* (1998) suggested causes may include predation on egg masses or disturbance of benthic communities by the recently introduced northern Pacific seastar (*Asterias amurensis*), habitat modification through increased siltation, heavy metal contamination or urban effluent. The Derwent is a heavily impacted estuary receiving contaminant inputs from a variety of sources including sewage treatment works, large industries (e.g., a paper mill and zinc refinery) and urban runoff. Sedimentation rates are high in certain localised areas within the estuary, particularly upstream of Hobart (Coughanowr, 1997). However, changes to sediment type within habitats previously occupied by *B. hirsutus* have not been investigated. Improvements have been documented in both heavy metal levels in biota within the Derwent over the last 20 years (Dineen and Noller, 1995) and in water quality over the last 10 years (Coughanowr, 1997) both as a result of decreased emissions from sewerage treatment plants and industries. However, heavy metal contamination within the estuary still remains a significant problem (Coughanowr, 1997) and the impact of this on *B. hirsutus* is unknown.

The possible role of *Asterias amurensis* in the decline of *B. hirsutus* has not yet been established, although the timing of its discovery and subsequent increase in abundance matches the mid to late 1980's period of decline in *B. hirsutus*. *A. amurensis* is now abundant in many areas where *B. hirsutus* was previously common. Small numbers of *A. amurensis* were present throughout the study area in 1996, but densities increased significantly in 1997. We did not observe any predatory loss of eggs during the study period, although only two (1996) egg masses were followed in detail. Although direct predation on egg masses was not observed, *A. amurensis* was observed feeding on the stalked ascidian (*Sycozoa* sp.) on two occasions. *Sycozoa* provided almost the only stable vertical



substrate for spawning within the survey area and, from our observations, probably the most common vertical substrate within this region of the Derwent. It is thus also possible that predatory loss of *Sycozoa* may impact *B. hirsutus* by reducing the available spawning substrate. Interestingly, *Sycozoa* were observed in low numbers in the core area during the 1997 spawning season when no egg masses were recorded. This coincided with a dramatic increase in the density of *A. amurensis*.

We hope that further monitoring of both this population and others subsequently discovered within the lower Derwent and adjacent Frederick Henry Bay will enable us to identify processes responsible for the decline in *B. hirsutus* and develop appropriate amelioration strategies.

**Acknowledgements.** - The following people have assisted us with the project: A. Beech, R. Bradford, K. Gowlett-Holmes, P. Hart, T. Lamb, S. Long, D. MacDonald, A. Morris, A. Phillips, J. Ross, J. Scott, C. Sutton and B. Wilson. We are grateful to the Tasmanian Aquaculture and Fisheries Institute for the use of their facilities and their input to the captive rearing component of the project. The work was supported by grants from Environment Australia (Endangered Species Program, Proj. No. 538 + 572), the Department of Primary Industry and Energy under the FISHCARE Program and was coordinated by the Spotted Handfish Recovery Team.

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## STATUS OF THE FRESHWATER FISHES OF THE PHILIPPINES

by

Emily CAPULI & Rainer FROESE (1)

FishBase records 271 fish species from fresh waters of the Philippines. One hundred twelve of these are primary freshwater species, 43 are secondary freshwater species, 81 are diadromous and 35 are introduced species. Thirty-two species are contained in the 1996 IUCN Red List, of which 22 are endemics, mainly cyprinids found only in Lake Lanao, Mindanao.

Species which are critically endangered are *Cephalakompsus pachycheilus*, *Hampala lopezi*, *Mandibularca resinus*, *Ospatulus truncatulus*, *Pandaka pygmaea*, *Puntius amarus*, *P. baoulan*, *P. clemensi*, *P. disa*, *P. flavifuscus*, *P. herrei*, *P. katolo*, *P. lanaoensis*, *P. manalak*, *P. tras* and *Spratellichthys palata*; endangered are *Ospatulus palaemophagus*, *Pristis microdon*, *P. pectinata* and *Scleropages formosus*, the last species needing further confirmation of its occurrence in the country. Vulnerable are *Puntius lindog*, *P. sirang*, *P. tumba*, *P. manguaoensis* and *P. hemictenus*; lower risk/near threatened are *Butis butis*, *Eleotris melanosoma*, *Glossogobius biocellatus*, *Papillogobius reichei* and *Redigobius bikolanus*; lower risk/Conservation dependant for *Mistichthys luzonensis*. *Puntius cataractae* is data deficient.

Twenty-eight species not reported to occur in other countries belong to families Ariidae (*Arius manillensis* and *A. magatensis*), Clupeidae (*Sardinella tawilis*), Cyprinidae (*Nematabramis verecundus*, *Puntius bantolanensis*, *P. montanoi*, *Rasbora philippina*, *R. punctulatus* and *R. taytayensis*), Eleotridae (*Bostrychus albooculata*, *B. expatria*, *Hypseleotris agilis*, *H. bipartita* and *Paloa polylepis*), Gobiidae (*Gobiopterus lacustris*, *G. stellatus*, *Mugilogobius montalbani*, *M. piapensis*, *Rhinogobius flavoventris*, *Tamanka cagayensis*, *T. maculata* and *T. siitensis*), Hemiramphidae (*Zenarchopterus cagayensis* and *Zenarchopterus cotnog*), Siluridae (*Hito taytayensis* and *Silurus palavanensis*) and Syngnathidae (*Microphis jagorii* and *M. pleurostictus*). Some of these possible endemics are used in the fishery and aquarium trade.

Of primary concern regarding migratory species are those involved in the « ipon » (gobiid) fisheries. Species of importance are *Eleotris melanosoma*, *Ophieleotris aporos*, *Awaous melanocephalus*, *Glossogobius giuris*, *G. celebius*, *Sicyopterus lacrymosus* and *Rhyacichthys aspro*. There are 50 other taxa belonging to families Apogonidae, Clupeidae, Engraulidae, Leiognathidae, and Silliganidae involved in this fishery which is mostly practiced at the river mouths of Abra, Abulug and Cagayan of Northern Luzon.

There are several factors effecting decrease in fish population and production in lakes, rivers and reservoirs: pollution (physical like siltation and chemical from industrial effluents, agricultural, and domestic wastes), major problem afflicting Laguna de Bay,

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near Manila; overfishing due to efficient fishing gears and illegal fishing practices as were once practiced in lakes Buhi and Bato and Caliraya in the Central Philippines; introductions of exotic species enhancing competition for food and space and predation, as in the case of the cichlid *Oreochromis mossambicus* preying on *Mistichthys luzonensis* in Lake Buhi and the eleotrid *Hypseleotris agilis* feeding on the endemic cyprinids of Lake Lanao; and, natural events like earthquakes and volcanic activities forming natural barriers and causing habitat destruction. At present, only *Sardinella tawilis* of Taal Lake and *Mistichthys luzonensis* of lakes Buhi, Bato and Manapao are protected under national laws.

There is an urgent need for a national fish survey to assess the current status of freshwater species in the wild, especially those which are commercially exploited. As a national effort to address these problems, the Philippine Council for Aquatic and Marine Research and Development (PCAMRD) in collaboration with other research institutions and universities formulated a five-year plan (1996-2000) to conduct stock assessment of fishes important to the fisheries and those that are endangered. Priority is given to taxonomic, ecological, developmental biology, life history and reproduction studies looking at appropriate management schemes and strategies such as zoning of specific areas; open and close seasons; establishment of sanctuaries; appropriate fishing practices and gear designs; and review of existing fishery laws for inland waters. Studies will include monitoring and evaluation of the environment through limnological surveys, fish productivity, and socio-economic issues. These will apply to both lakes and reservoirs, particularly to Laguna de Bay, lakes Taal, Buhi and Bato in Luzon, Lake Bito of Leyte in the Visayas, and lakes Lanao and Sebu in Mindanao. Introductions of new exotic species should be done with extreme caution so as not to have adverse impact to the environment and to resident species. For these management and conservation effort to work, mutual support of both the government and the community or resource holders is needed.

The Philippines has been identified as one of the Asian countries which have relevant data; however, there are geographical gaps which require extensive additional field work. Databases may well serve as a tool for documenting the valid species, occurrence, status, and other biological information that can help monitor, manage, and conserve inland fish resource. In addition, given the lack of formally trained fish taxonomists in the Philippines, there is also an urgent need to create taxonomic positions and to secure the assistance of taxonomists from other countries. Unless drastic steps are taken to conserve the native freshwater fish resources of the Philippines, these valuable resources will be lost.

## RÉSUMÉ. - Statut des poissons d'eau douce des Philippines

La base de données FishBase contient 271 espèces de poissons des eaux douces des Philippines. Parmi celles-ci, 112 sont primitivement d'eau douce, 43 sont secondairement d'eau douce, 81 sont diadromes et 35 ont été introduites. La Liste Rouge de 1996 recense 32 espèces, dont 22 endémiques, principalement des Cyprinidae du lac Lanao de Mindanao.

Les espèces sérieusement en danger sont les suivantes: *Cephalakompsus pachycheilus*, *Hampala lopezi*, *Mandibularca resinus*, *Ospatulus truncatulus*, *Pandaka pygmaea*, *Puntius amarus*, *P. baoulan*, *P. clemensi*, *P. disa*, *P. flavifuscus*, *P. herrei*, *P. katolo*, *P. lanaoensis*, *P. manalak*, *P. tras* et *Spratellichthys palata*. Les espèces en danger sont:

*Ospatulus palaemophagus*, *Pristis microdon*, *P. pectinata* et *Scleropages formosus*; la présence de cette dernière doit être confirmée. Les espèces vulnérables sont: *Puntius lindog*, *P. sirang*, *P. tumba*, *P. manguaoensis* et *P. hemictenus*. Les espèces à faible risque ou susceptibles d'être menacées sont: *Butis butis*, *Eleotris melanosoma*, *Glossogobius bio-cellatus*, *Papillogobius reichei* et *Redigobius bikolanus*; *Mistichthys luzonensis* est dépendante des mesures de conservation qui pourront être prises et les données manquent pour *Puntius cataraetae*.

À ce jour, les 28 espèces des familles suivantes ne sont connues qu'aux Philippines: Ariidae (*Arius manillensis* et *A. magatensis*), Clupeidae (*Sardinella tawilis*), Cyprinidae (*Nematabramis verecundus*, *Puntius bantolanensis*, *P. montanoi*, *Rasbora philippina*, *R. punctulatus* et *R. taytayensis*), Eleotridae (*Bostrychus albooculata*, *B. expatria*, *Hypseleotris agilis*, *H. bipartita* et *Paloa polylepis*), Gobiidae (*Gobiopterus lacustris*, *G. stellatus*, *Mugilogobius montalbani*, *M. piapensis*, *Rhinogobius flavoven-tris*, *Tamanka cagayensis*, *T. maculata* et *T. siitensis*), Hemiramphidae (*Zenarchopterus cagayensis* et *Z. cotnog*), Siluridae (*Hito taytayensis* et *Silurus palavanensis*) et Syngnathidae (*Microphis jagorii* et *M. pleurostictus*). Certaines de ces espèces, potentiellement endémiques, sont exploitées par les pêcheries et le commerce des poissons d'aquarium.

En ce qui concerne les espèces migratrices, celles qui sont exploitées accessoirement par les pêcheries d'« ipon » (Gobiidae) sont les plus menacées, notamment: *Eleotris melanosoma*, *Ophieleotris aporos*, *Awaous melanocephalus*, *Glossogobius giuris*, *G. celebius*, *Sicyopterus lacrymosus* et *Rhyacichthys aspro*. Une cinquantaine d'autres espèces, appartenant aux familles des Apogonidae, Clupeidae, Engraulidae, Leiognathidae et Silliganidae, est concernée par ces pêcheries qui sont pratiquées principalement dans les estuaires des rivières Abra, Abulug et Cagayan dans la partie nord de Luzon.

Plusieurs paramètres sont responsables de la diminution des populations de poissons et de la production des lacs, rivières et réservoirs: la pollution (physique telle que la sédimentation et les rejets des effluents chimiques industriels et des déchets agricoles et domestiques), tel est le cas de la lagune de Bay, près de Manille, gravement touchée; la surpêche due à la grande efficacité des engins de pêche et le braconnage tel qu'il a été pratiqué dans les lacs Buhi, Bato et Caliraya dans la partie centrale des Philippines; l'introduction d'espèces exotiques augmentant la compétition pour la nourriture et l'espace, comme par exemple le Cichlidae *Oreochromis mossambicus* qui se nourrit de *Mistichthys luzonensis* dans le lac Buhi et l'Eleotridae *Hypseleotris agilis* qui se nourrit sur les cyprins endémiques du lac Lanao; enfin, les événements naturels tels que les tremblements de terre et l'activité volcanique qui peuvent former des barrières naturelles et provoquer la destruction des habitats. A présent, seule la sardinelle *Sardinella tawilis* du lac Taal et *Mistichthys luzonensis* des lacs Buhi, Bato et Manapao sont protégées par des lois nationales.

Il est urgent d'entreprendre un recensement national des poissons pour définir le statut des espèces d'eau douce, particulièrement celui des espèces exploitées commercialement. Le conseil des Philippines pour la recherche marine et aquatique et le développement (PCAMRD), en collaboration avec d'autres instituts de recherche et des universités, a engagé un effort national en établissant un plan quinquennal (1996-2000) pour mettre en oeuvre une gestion des stocks de poissons importants pour la pêche et pour les espèces en danger. La priorité a été donnée aux recherches relatives à la taxinomie, l'écologie, la biologie du développement, les cycles biologiques et la reproduction des espèces, afin de déterminer les solutions et les stratégies appropriées, comme par exemple la détermina-

tion de zones spécifiques, l'établissement de périodes d'ouverture et de fermeture de la pêche, la création de sanctuaires, l'amélioration des méthodes et des engins de pêche, et la revision des lois existantes relatives aux pêches continentales. Ces études incluront la gestion de l'environnement et son évaluation par un suivi limnologique, de la productivité et des facteurs socio-économiques. Elles concerneront les lacs et les réservoirs, particulièrement la lagune de Bay, les lacs Taal, Buih et Bato à Luzon, le lac Bito de Leyte dans les Visayas et les lacs Lanao et Sebu de Mindanao. Les introductions de nouvelles espèces exotiques devront être faites avec d'extrêmes précautions de façon à éviter tout impact néfaste sur l'environnement et les espèces locales. Pour que ces efforts de gestion et de conservation puissent être efficaces, le support conjoint du gouvernement, des communautés et des propriétaires est nécessaire.

Les Philippines sont reconnues comme l'un des pays asiatiques ayant des données fiables, mais il y a des lacunes géographiques qui nécessitent un travail de terrain complémentaire. Les bases de données peuvent être utilisées comme outils documentaires en intégrant l'information sur le statut des espèces, leur distribution et leur biologie, et contribuer ainsi à la gestion et à la conservation des ressources continentales. De plus, étant donné le manque d'ichthyologistes expérimentés aux Philippines, il est urgent de créer des postes et de s'assurer l'aide de taxinomistes d'autres pays. Si on ne prend pas ces mesures drastiques pour conserver les ressources ichthyologiques continentales des Philippines, ces dernières seront perdues.

## DOCUMENTING THE STATUS OF FRESHWATER FISH INTRODUCTIONS IN OCEANIA

by

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**ABSTRACT.** - Over 220 freshwater fish introductions of 102 species have been recorded in Oceania. Approximately three quarters of these introductions have established feral populations. The three most often introduced species were *Oreochromis mossambicus* (9%), *Gambusia affinis* (7%) and *Poecilia reticulata* (5%). The major reasons for introducing alien fish species to the region include: ornamental use (17%), aquaculture (16%), and biological control of mosquitoes (12%). However, many of the records (24%) did not specify the reasons for the transfers (category "unknown"). Available data indicate that the number of introduced species now accounts for a major fraction of the number of freshwater fish species in some Pacific islands. For the seven most introduced species, ecologically or economically adverse effects have been reported from at least one of the countries in the area. Implications of the changes in the freshwater ecosystems, as caused by some of these introductions, are presented and discussed.

**RÉSUMÉ.** - Bilan des introductions de poissons d'eau douce en Océanie.

Plus de 220 introductions de poissons d'eau douce appartenant à 102 espèces ont été observées en Océanie. Près des trois quarts ont formé des populations sauvages. Les espèces le plus souvent importées sont *Oreochromis mossambicus* (9%), *Gambusia affinis* (7%) et *Poecilia reticulata* (5%). L'importation d'espèces non autochtones est principalement due à l'aquariophilie (17%), l'aquaculture (16%) et au contrôle biologique des moustiques (12%). La proportion d'introductions non justifiées (24%) est la plus élevée. Des données récentes montrent que les populations actuelles de certaines îles du Pacifique sont majoritairement constituées de poissons importés. L'article traite de l'impact de ces introductions sur le fonctionnement des écosystèmes.

**Key-words.** - Freshwater fishes, Oceania, Introduced species, Ecological impacts.

Freshwater fish have been widely introduced throughout the world: these introductions have been compiled in the Introductions table of FishBase (Froese and Pauly, 1997). In Oceania, such introductions have been made primarily for the presumed benefit of humans, e.g., for food, for ornamental use and for biological control. However, adverse ecological impacts can also arise from the use of introduced species. Welcomme (1988) categorized problematic effects of fish introductions into: 1) predation and competition; 2) hybridization; 3) introduction of disease and parasitic organisms; 4) crowding and stunting; and 5) environmental changes, such as the eutrophication of water.

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The present paper is ICLARM Contribution n°1419.

Alien species will almost always escape to the wild and, once established, cannot usually be eradicated. There are, however, few reports on the impacts of alien species on

Table I. - Freshwater fish introductions to Oceania and their establishment in the wild.

Reason for the introductions	Numbers of introductions recorded	Number of records of establishment in the wild as a result of these introductions
Ornamental use	38	32
Aquaculture	37	25
Mosquito control	27	27
Unknown	54	33
Others	68	58

Table II. - Countries in Oceania with alien freshwater fishes. The data presented refer to multiple species. Freshwater fishes include the diadromous species. \* Numbers in parentheses give the number of endemic freshwater species.

Country	Introduction records	Number established in the wild	Total number of freshwater species* (FishBase 97)	% of established alien species
American Samoa	4	4	18	22
Australia	30	26	268 (120)	10
Cook Islands	4	3	6	50
Fiji	27	14	45 (1)	31
Guam	22	14	15	93
Hawaii	58	45	53 (3)	85
Kiribati	3	2	3	67
Marshall Islands	1	1	4	25
Micronesia	2	2	18 (1)	11
Northern Marianas	4	3	12	25
Nauru	1	1	2	50
New Caledonia	10	9	80	11
New Zealand	28	25	49 (22)	51
Niue	1	1	2	50
Palau	4	4	34 (1)	12
Polynesia French	3	3	29	10
Samoa	5	5	31	16
Solomon Islands	2	2	24	8
Tahiti	9	5	27	19
Tonga	1	1	2	50
Tuvalu	1	1	1	100
Vanuatu	1	1	22	5
Wallis and Futuna	3	3	4	75
Total	224	175	749	



fisheries and aquatic environments in Oceania. This may be because there were no noticeable impacts, or no baseline studies of the pre-introduction were done to assess accurately any impact due to the introduction, or there was no assessment and monitoring of the introduction. This knowledge gap is alarming, given the high degree of establishment of introduced fish species in the wild.

This paper presents the status of freshwater fish introductions to Oceania (taken here as the tropical island states of the central Pacific, Australia and New Zealand, but excluding Papua New Guinea) and reviews their ecological effects and the biological characteristics of the most commonly introduced species. The data presented in this paper were mainly derived from Maciolek (1984), Welcomme (1988), Eldredge (1994) and FishBase 97 (Froese and Pauly, 1997).

### Status of freshwater fish introductions to Oceania

There have been over 220 freshwater fish species introduced to Oceania. Over 75% of the introduced species have become established in the wild, 13% have failed to become established, and information is lacking for the remaining 12%. The most common reasons for the introductions have been: ornamental use, 17%; aquaculture, 16%; and mosquito control, 12%. The reasons for 24% of the freshwater fish introductions are unknown. Table I summarizes the data available and table II shows the extent of establishment of alien freshwater fish species in Oceania, by country.

### Ecological effects of these introductions

Of the 11 most commonly introduced species, seven are considered to have had adverse effects in at least one country; i.e., they are considered undesirable, a nuisance, a noxious species, or a pest (Table III). Table IV summarizes the different ecological effects of freshwater fish introductions to Oceania. Competition, crowding and stunting were the main effects reported. *Oreochromis mossambicus*, has been spread around the tropical world for aquaculture, but has often not produced the desired results and is now considered a nuisance in many regions (like South Asia). Their populations have undergone rapid expansion, usually accompanied by stunting and early maturity at a small size. As a result of the introduction, many waterbodies contain numerous small and stunted

Table III. - Freshwater fish species commonly introduced to Oceania.

Species	Numbers of introduction records and countries where they have adverse ecological effects
<i>Oreochromis mossambicus</i>	21 (Australia, Hawaii, Kiribati, Nauru and Solomon Is.)
<i>Gambusia affinis</i>	15 (Australia, New Zealand)
<i>Poecilia reticulata</i>	11 (Australia)
<i>Cyprinus carpio</i>	7 (Australia)
<i>Poecilia mexicana</i>	6
<i>Xiphophorus helleri</i>	6 (Australia)
<i>Carassius auratus</i>	6 (Australia)
<i>Oncorhynchus tshawytscha</i>	5 (Australia)
<i>Salmo salar</i>	5
<i>Micropterus salmoides</i>	5
<i>Tilapia zillii</i>	5

Table IV. - Known ecological effects of freshwater fish species introduced to Oceania.

Species	Ecological effect
<i>Ameiurus nebulosus</i>	New Zealand - Considered a nuisance because it competes with eel (Welcomme, 1988; McDowall, 1990; Csavas, 1995).
<i>Carassius auratus</i>	Australia - Instrumental in the entry of the goldfish ulcer disease, which has recently spread rapidly to wild stocks (McKay, 1984; Arthington, 1989).
<i>Cyprinus carpio</i>	Australia - Although regarded as a pest by some, it forms the basis for fisheries and is thought by others to be a valuable addition to the aquatic fauna. The diet of this fish overlaps with that of several endemic fishes, such as bony bream ( <i>Nematolosa erebi</i> ), catfish ( <i>Tandanus tandanus</i> ), silver perch ( <i>Bidyanus bidyanus</i> ), Australian smelt ( <i>Retropinna semoni</i> ), carp gudgeon ( <i>Hypseleotris klunzingeri</i> ) and flat headed galaxias ( <i>Galaxias rostratus</i> ) (McKay, 1984; Arthington, 1989).
<i>Gambusia affinis</i>	Australia - Considered a noxious species and its possession is prohibited (Allen, 1991). New Zealand - Often described as aggressive, fin-nipping and egg-eating predators; their increasing spread could be a cause for concern with regard to the black mudfish ( <i>Neochanna diversus</i> ), which is already much reduced in range (McDowall, 1990).
<i>Gambusia holbrocki</i>	Australia - Partly responsible for the decline of several endemic species. Also attacks the eggs and fry of important sport fishes.
<i>Misgurnus anguillicaudatus</i>	Australia - The species was banned from import as an aquarium fish due to its documented habits (Burchmore <i>et al.</i> , 1989; McKay, 1989).
<i>Oreochromis mossambicus</i>	Australia - Has hybridized with <i>O. urolepis hornorum</i> and possibly with other tilapia as well. Nothing is yet known about the physiological and ecological characteristics and consequent potential for the spread of this mixed strain. Has been declared as a noxious species in Queensland with heavy penalties for translocation and cultivation (McKay, 1984; Arthington, 1989). Hawaii - Suspected of reducing the population of the valuable mullet, <i>Mugil cephalus</i> , by competing aggressively for the same food source of soft algae and detritus (Randall, 1987). Kiribati - A decrease of mullet, bonefish and milkfish was observed after its establishment to the Fanning Atoll (Eldredge, 1994). Attempts to eradicate stunted populations, which hinder the development of milkfish culture, proved unsuccessful (Lobel, 1980). Nauru - Stunted tilapia in inland ponds and lagoons destroyed traditional culture of milkfish and attempts to eradicate tilapia were unsuccessful. Milkfish, a highly valued food used for special occasions and ceremonies in Nauru had to be imported from Guam or Kiribati (Nelson and Eldredge, 1991; Eldredge, 1994). Solomon Islands - The extinction of two duck species ( <i>Anas superciliosa</i> and <i>A. gibberifrons</i> ) is speculatively attributed to this introduction (Nelson and Eldredge, 1991; Eldredge, 1994).
<i>Perca fluviatilis</i>	Australia - Has displaced native species. Feeds selectively on small endemic fishes and fish fry and may have affected the numbers of galaxiids, pygmy perch, <i>Nannoperca australis</i> , and the golden perch, <i>Macquaria ambigua</i> in southern rivers (McKay, 1984; Arthington, 1989).
<i>Poecilia latipinna</i>	Australia - The spread of this species could have an adverse effect on endemic fishes. Regarded as undesirable. (McKay, 1984; Arthington, 1989). Hawaii - Mainly introduced to control mosquitoes, later found to be a failure; generally regarded as a pest (Randall, 1987).
<i>Salmo trutta</i>	Australia - May have caused the decline of the Tasmanian mountain shrimp <i>Anaspides tasmaniae</i> , and has eliminated or reduced several <i>Plecoptera</i> and <i>Trichoptera</i> in Victorian streams (McKay, 1984; MacKinnon, 1987; Arthington, 1989).
<i>Scardinius erythrophthalmus</i>	New Zealand - Considered as a noxious species (McDowall, 1984, 1990).
<i>Xiphophorus helleri</i>	Australia - Its presence in large numbers seem to depress endemic fish populations (McKay, 1984; Arthington, 1989).

tilapia that compete with native species for living space and food. Similarly, introductions of *Poecilia latipinna* for mosquito control are now often regarded as failures and this species is now considered a pest (McKay, 1984; Randall, 1987; Arthington, 1989). Of the 224 freshwater fish introductions to Oceania, 24 reported adverse ecological effects and one beneficial effect. Moreover, there were 20 beneficial and eight adverse socioeconomic effects (Froese and Pauly, 1997).

### What influences the establishment of introduced species?

Although it is often difficult to predict whether an introduction will lead to self-sustaining populations (Townsend, 1996), some general conditions favoring establishment include, *inter alia*: 1) altered or disturbed habitats, 2) habitats that have low biological diversity, 3) when the introduced species has wide environmental tolerances, and 4) when the species has certain life history characters, such as high fecundity, short generation time and simple breeding/spawning requirements (Balz, 1991; Balz and Moyle 1993). Moyle and Light (1996) pointed out that successful establishment depends on the interaction of the abiotic and biotic environment, and on the biological characteristics of introduced species.

The three most commonly introduced species to Oceania (*Oreochromis mossambicus*, *Gambusia affinis* and *Poecilia reticulata*) possess most of the biological characteristics needed to colonize new habitats (Marshall, 1995) (Table V). Their temperature ranges are similar to those of the recipient countries. Their ability to survive and thrive in various habitats, from clear running streams to heavily polluted waters, is another plus. In addition, *O. mossambicus* and *P. reticulata* can tolerate high salinity. The trophic plasticity of *O. mossambicus* (Maiti and De Silva, 1985) ensures its survival in water systems which may be unfavorable to other species with less flexible feeding habits. All three species have early maturation, year-round spawning and no need for special spawning habitats, thus substantiating the idea that an alien species with unspecialized reproductive behavior and high reproductive output is more likely to become established than a species with strict biological and environmental requirements.

Table V. - Some biological characteristics of *Oreochromis mossambicus*, *Gambusia affinis* and *Poecilia reticulata* that are relevant for successful introductions.

Biological characteristics	<i>Oreochromis mossambicus</i>	<i>Gambusia affinis</i>	<i>Poecilia reticulata</i>
Maximum length	39.0 cm (SL)	4.0 cm (TL)	3.5 cm (TL)
Habitat	Temperature range: 8-42°C; demersal; can be found in various habitats.	Temperature range: 18-24°C; benthopelagic.	Temperature range: 18-28°C; pelagic; found in various habitats and has a wide salinity range.
Food	Omnivorous; feeds on almost anything from algae to insects.	Feeds on zooplankton, small insects and detritus.	Feeds on zooplankton, small insects and detritus. Mainly carnivorous.
Maturity	About 1-2 years in native range, but may be only 2-3 months elsewhere and under certain conditions.	Several months only.	About 2-3 months in aquaria.
Spawning	No obvious seasonal peak.	No obvious seasonal peak.	No obvious seasonal peak.
Reproduction	Mouthbrooder, up to 300 eggs.	Livebearer, 40-60 offspring every 5-8 weeks.	Livebearer, 20-40 offspring.

### Precautionary approach

Oceania comprise about 5% of the world's annual internal renewable water resource (Gleick, 1993) and only a mere 2.44% of total freshwater fish species (McAllister *et al.*, 1997). The limited choice available to people looking to use fish for various ends (food, etc.) have led them to bring in exotics. In Oceania, native freshwater fishes are few, but may represent valuable aquatic biodiversity not found anywhere else, i.e., the region has a high rate of endemism such as that often found on islands (Table II). In several countries more than half of the freshwater fishes are alien species, with impacts on the native fauna still imperfectly known. There is a growing appreciation on a global level that native aquatic resources should be protected, conserved and sustainably utilized.

Introductions of freshwater fish that are well planned, where a thorough appraisal of possible impacts has been undertaken, and where monitoring and evaluation programmes are in place to judge the real impacts, can benefit local communities by increasing production value from inland waters (Coates, 1995). Such planning should also involve contingency plans or remedial measures in the event that adverse effects are observed following an introduction (However, as pointed out earlier, eradication of an alien species once it has become established is difficult). This in effect, is part of a precautionary approach to species introductions (FAO, 1995; Bartley and Minchin, 1996). Codes of practice and guidelines have been established to assist in the use of introduced species and, perhaps, represent one of the best means of precaution (ICES, 1995; Bartley *et al.*, 1996). Thorough ecological appraisals, including the presence of vacant niches and a comparison of the ecological characteristics of the introduced species to natives will be essential in this approach (Coates, 1993). In addition, databases such as FishBase can provide information on the effects that an introduction has had in other countries where it has been introduced. Also part of a precautionary approach is the assumption that introduced species will escape into the wild. Whether they become established or not should be assessed *a priori* by desk studies and then followed-up with monitoring if the introduction is actually made or approved.

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## IS THE MOSQUITOFISH, *GAMBUSIA HOLBROOKI* (POECILIIDAE), A MAJOR THREAT TO THE NATIVE FRESHWATER FISHES OF SOUTH-WESTERN AUSTRALIA ?

by

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**ABSTRACT.** - Although there is much anecdotal evidence to suggest that *Gambusia holbrooki* is agonistic towards similar-sized fishes, the effect of this behaviour, e.g., fin nipping and/or increased mortality, on Australian species has never previously been quantified. We have therefore, a) determined the extent and effects of the agonistic behaviour of *G. holbrooki* towards the pigmy perch *Edelia vittata* in controlled environments, b) quantified the incidence of fin damage in populations of native fishes from lentic systems representative of those found in south-western Australia, c) compared the distribution and abundance of *E. vittata* in a rehabilitated lentic system that contains large numbers of *G. holbrooki* with these parameters in a more natural system that is also infested with *G. holbrooki*, and d) described the association between native species, *G. holbrooki* and habitat type in 163 lentic systems (representative of the major lentic habitat types) in south-western Australia. Finally, the results from the above components have been used to determine if *G. holbrooki* poses a threat to populations of native fishes in south-western Australia and, if so, how this threat may be ameliorated.

**RÉSUMÉ.** - *Gambusia holbrooki* (Poeciliidae) est-elle une menace pour les poissons indigènes des eaux douces du sud-ouest de l'Australie ?

Bien qu'il y ait de nombreux indices suggérant que la présence de la gambusie, *Gambusia holbrooki*, dans les eaux douces du sud-ouest de l'Australie, est néfaste pour les poissons indigènes de taille semblable par son comportement particulier, notamment le grignottage des nageoires, ce phénomène n'avait jamais été étudié quantitativement. Nous avons donc: a) déterminé les effets et l'étendue du comportement agressif de *G. holbrooki* envers la perche naine *Edelia vittata* en milieu contrôlé, b) quantifié l'impact de la détérioration des nageoires sur les populations de poissons indigènes dans les systèmes lacustres représentatifs de ceux du sud-ouest de l'Australie, c) comparé la distribution et l'abondance de *E. vittata* dans un milieu lacustre réhabilité contenant un grand nombre de *G. holbrooki* et dans un système plus naturel qui était également colonisé par *G. holbrooki*, et d) décrit l'association entre les espèces indigènes, *G. holbrooki* et le type d'habitat dans 163 systèmes lacustres (représentatifs de la plupart des types d'habitats) du sud-ouest de l'Australie. Finalement, les résultats obtenus ont été utilisés pour déterminer si *G. holbrooki* est une menace pour les populations de poissons indigènes du sud-ouest de l'Australie, et dans l'affirmative, comment cette menace pouvait être réduite.

**Key-words.** - Poeciliidae, *Gambusia holbrooki*, Mosquitofish, S.W. Australia, Freshwaters, Native fishes, Introduced fishes, Threats, Fin nipping, Agonistic behaviour.

Many authors (e.g., Jackson and Williams, 1980; Fletcher, 1986; Arthington, 1989, 1991; Crowl *et al.*, 1992) have suggested that introduced fish species may result in the decline or displacement of native fish populations from Australian waterbodies

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through competition for limited resources; predation upon adults, juveniles, larvae or eggs of native species and the introduction of diseases and parasitic organisms. For example, Crowl *et al.* (1992) cite 14 studies attributing the displacement of nine species of native fish from much of their natural ranges through competition for both food and/or space and direct predation by introduced salmonids, while the introduced fish louse (*Argulus* spp.) and the anchor worm (*Lernaea cyprinacea*) have parasitised several native fish species (Roberts, 1978; Wager and Jackson, 1993).

Approximately 20 species (five families) of introduced freshwater fish have established self-maintaining populations in Australia (Fletcher, 1986; Arthington, 1991). About half of these introductions are the result of accidental releases of aquarium species (Poeciliidae, Cichlidae), while the remainder were deliberately introduced for either sport or food (Salmonidae, Cyprinidae and Percidae), ornamental purposes (Cyprinidae) or as a biological control agent (*Gambusia holbrooki*, Poeciliidae) (McKay, 1984; Fletcher, 1986; Arthington, 1989, 1991). *Gambusia holbrooki* is now the most widely distributed freshwater fish in Australia (Harris, 1984; Lloyd *et al.*, 1986) and has been a particularly successful coloniser of disturbed and rehabilitated lentic habitats (Courtenay *et al.*, 1974; McKay, 1984; Lloyd *et al.*, 1986). A large body of circumstantial evidence suggests that *G. holbrooki* preys on the eggs and larvae of, and also exhibits agonistic behaviour, such as fin and scale nipping, towards, similar sized fishes (see McDowall, 1980; Lloyd, 1990; Howe *et al.*, 1997 and references therein). However, although *G. holbrooki* has been shown to deleteriously affect the reproductive success of the blue-eye *Pseudomugil signifer* in controlled environments (Howe *et al.*, 1997), the extent and severity of agonistic interactions have never been measured, while no studies have demonstrated whether *G. holbrooki* alone could displace a population of native Australian fish.

In less than 100 years agricultural practices and urbanisation have resulted in the loss of many freshwater habitats in south-western Australia, while continuing deforestation and draining of wetlands for agriculture are causing alterations to inflow, salinisation, siltation and eutrophication of freshwaters in the lower south-west. During this period *Perca fluviatilis*, a large piscivore, and *G. holbrooki* have become well established in the region. Thus, the 10 species of native fishes, of which eight are endemic, are vulnerable to the continuing loss or alteration of habitat and to the introduction of non-native species. Furthermore, with the exception of the freshwater catfish (*Tandanus bostocki*), none of the native species typically exceeds 140 mm in total length (Morgan *et al.*, 1998) and so could be particularly vulnerable to agonistic behaviour by *G. holbrooki*. Of the small species, the salamanderfish (*Lepidogalaxias salamandroides*) and black-stripe minnow (*Galaxiella nigrostriata*) are essentially restricted to ephemeral pools in the small area of peat flats along the south coast, while the mud minnow (*Galaxiella munda*) and Balston's pigmy perch (*Nannatherina balstoni*) are generally associated with the small streams and permanent pools of the peat flat region, respectively (Morgan *et al.*, 1998). The spotted minnow (*Galaxias maculatus*) and trout minnow (*Galaxias truttaceus*) are found in only a few streams and lakes to the east of the peat flats but are widespread in south-eastern Australia (Morgan *et al.*, 1998). The remaining species, the western minnow (*Galaxias occidentalis*), nightfish (*Bostockia porosa*) and western pigmy perch (*Edelia vittata*) are found in all types of waterbodies and are widespread throughout south-western Australia (Morgan *et al.*, 1998). As the last of these species not only grows to a similar size, but also has a similar diet to that of *G. holbrooki* (Pen and Potter, 1991; Hambleton *et al.*, 1996; Pusey and Bradshaw, 1996), it is likely that any interactions between *G. holbrooki* and *E. vittata* will be stronger than between *G. hol-*



*brookii* and the other native species. It should be noted that Pen and Potter (1991) reported no evidence of agonistic behaviour by *G. holbrooki* towards *E. vittata* or the other native fishes in the Collie River to the south of Perth. Indeed these workers considered that *G. holbrooki* posed little, if any, threat to the native fishes in lotic systems of south-western Australia. However, Morgan *et al.* (1996) noted that native fishes were absent in many of those lentic water bodies of the region that contained *G. holbrooki* or, when present, often exhibited fin damage which was possibly attributable to the agonistic behaviour of *G. holbrooki*. Morgan *et al.* (1996) also noted that in an artificial wetland that had been colonised by *G. holbrooki*, native fish were only ever abundant in those waterbodies that contained large amounts of cover.

Thus, the objectives of this study were to:

- 1) Determine and quantify the extent and effects of the agonistic behaviour of *G. holbrooki* towards *E. vittata* in controlled environments;
- 2) Determine the incidence of fin damage in populations of native fishes in the lentic systems of south-western Australia;
- 3) Describe and compare the distribution and abundance of *E. vittata* in a rehabilitated lentic system that contains large numbers of *G. holbrooki* with these parameters in a more natural system that is also infested with *G. holbrooki*;
- 4) Determine the association between native species, *G. holbrooki* and habitat type;
- 5) From the above, determine if *G. holbrooki* poses a threat to populations of native fishes in south-western Australia and, if so, how this threat can be minimised.

## MATERIALS AND METHODS

### Interaction between *Gambusia holbrooki* and *Edelia vittata* in tanks

Six 1.8 m diameter fibreglass tanks were filled with water to a depth of 0.6 m (volume ~1.6 m<sup>3</sup>); 500 g of salt was added and the water was then allowed to 'age' for at least two weeks. Sixteen *E. vittata*, taken randomly from a single wild population (25–56 mm total length) that had not been exposed to *G. holbrooki*, were then placed in each tank. Equal numbers of male and female *G. holbrooki* were introduced to tanks three days later at treatments of 0, 16 and 32 fish per tank, with each treatment being replicated twice. Fish were fed a combination of live zooplankton, floating fish pellets and fish flakes twice each day. Thus, food was considered not to have been limiting to either species and, as such, any fin damage could be attributed to agonistic behaviour of *G. holbrooki*. Tanks were checked twice each day and any dead fish were removed. After eight weeks, all fish from each tank were anaesthetised and examined for fin damage. The caudal, 2nd dorsal, anal, pectoral and pelvic fins were each given a relative fin damage score of 0, 1, 2 or 3; with 0 being a fin with no damage and 3 being a fin with major damage. The total fin damage score for each fish is therefore the score of damage to all fins, and is scored out of a possible 21. Each treatment was replicated twice.

The level of fin damage in individuals of *E. vittata*, in relation to the final densities of *G. holbrooki* in the six tanks, were also compared (see results for rationale). From this, the six treatments of *G. holbrooki* (0, 0, 3.2, 7.6, 11.4 and 18.4 fish/m<sup>3</sup>) were taken as being fixed factors and a Model I test for linear regression (Sokal and Rohlf, 1969) was used to compare the fin damage scores of individual *E. vittata* (from each treatment of *G. holbrooki*) against the independent treatments of *G. holbrooki*.

### Interaction between wild populations of *Gambusia holbrooki* and native species

One hundred and sixty three lentic waterbodies in the south-western corner of Australia were sampled for fish using seine, scoop and larval nets, fish traps and electrofishing gear, see Morgan *et al.*, (1998) for details of sampling sites and methodology. All fish from the RGC system (see below) were measured and had their fin damage scored as described above. A test for linear regression was employed to ascertain whether there was a relationship between individual fin damage and the total length of *E. vittata* from the RGC system. However, as the native fish from the other systems were to be returned to the water alive, and as sampling was undertaken between late spring and early autumn when air temperatures were often above 35°C, the damage to only the caudal fin was recorded for a maximum of 100 representatives of each species in each sample. Furthermore, as sampling was conducted a maximum of about six months after the native species had spawned we were able to confidently assign individuals to one of two groups, i.e., those fish that were the result of that years spawning and were therefore only a few months old and those fish that were the result of previous years spawning and were therefore at least one year old. Thus, the amount of damage to the caudal fin in 'young' fish could be compared to that in older fish.

### Distribution of *Gambusia holbrooki* and *Edelia vittata* in rehabilitated and natural wetlands

The RGC Wetland Centre is a rehabilitated mineral sands complex and comprises 17 mostly interconnected pools/lakes. The majority of these pools contain some emergent vegetation but little or no submerged aquatic vegetation or other cover, whereas, in addition to emergent vegetation, one shallow pool contains dense beds of submerged aquatic plants. It should be noted that, much of the fringing vegetation is only inundated with water during the wetter (winter/spring) months. The drains connecting the pools and that which leaves the wetlands include three main types of habitat, i.e., sections containing small boulders and submerged timber and/or macrophytes, sections containing small boulders but without submerged timber and/or macrophytes and finally sections that are devoid of any such cover.

Red Lake is a natural wetland system that comprises a main lake, a series of associated marginal pools and several drains that supply water to a nearby peat mine. Although the marginal pools and drains are connected to the main lake in winter they become isolated as water levels fall in the very dry summers that are characteristic of south-western Australia. Red Lake is surrounded by deep stands of emergent vegetation and has a substrate that varies from bare sand to organic matter covered with submerged aquatic plants and snags, while the associated pools contain no submerged vegetation or other cover. Furthermore, between late spring/early summer to autumn water levels fall and thereby expose any emergent vegetation in the associated pools. With the exception of one short section that contains a few small boulders, the drains between Red Lake and the peat mine are barren canals.

For comparative purposes a series of small pools in a wetland system that is adjacent to, but not connected with, Red Lake were also sampled. These pools have a similar morphology to the small marginal pools connected to Red Lake, i.e., no submerged cover and fringing vegetation only inundated in winter/early spring, but contain no *G. holbrooki*.

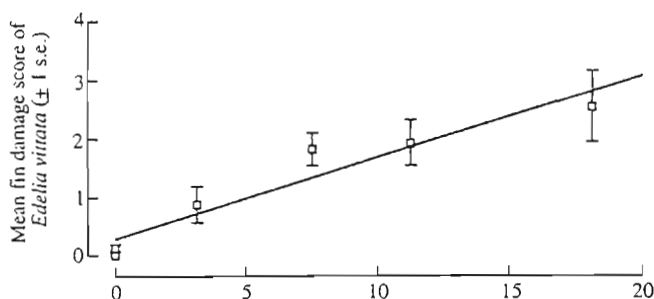


Fig. 1. - The relationship between fin damage of *Edelia vittata* (western pygmy perch) and the density of the mosquito fish, *Gambusia holbrooki*, in controlled tank experiments.

Sampling was carried out in late spring/early summer and mid-autumn (i.e., at a time when populations of both native species and *G. holbrooki* are at their highest) in all waterbodies within the artificial wetland and in areas of the lake with and without submerged plants or cover and also in the pools and drains. All sampling was carried out in the daylight hours using collapsible fish traps of 2 mm mesh, measuring 25x25x40 cm. A minimum of four traps were used in each waterbody/habitat type, each trap was baited with chicken pellets, placed in approximately 40 to 60 cm of water and retrieved after one hour. Relative abundance is estimated as the mean number of fish caught per trap per hour. N.B. The soft substrate in the RGC Wetlands prevented the use of seine nets or electrofishers.

One-way analyses of variance (ANOVA) were used to ascertain whether the relative abundance of *E. vittata* differed significantly amongst sites/habitat types. As Cochran's C test showed that these data were heteroscedastic, they were log-transformed [ $\log_{10}(N+1)$ ]. As Cochran's C test showed that the data were still heteroscedastic, the conclusions from the results of ANOVAs have concentrated on those cases where the significance levels were  $< 0.01$ . When ANOVA showed a significant difference, an *a posteriori* Scheffé test was then used to determine which means were significantly different at the 0.05 level of probability.

#### The association between native species, *Gambusia holbrooki* and habitat type

At each of the 163 lentic water bodies sampled in south-western Australia the species present and habitat characteristics, i.e., the presence of cover (submerged aquatic vegetation, sunken timber and/or boulders) and whether the waterbodies were permanent or ephemeral were recorded.

## RESULTS

#### Interaction between *Gambusia holbrooki* and *Edelia vittata* in tanks

On average, 23% of *E. vittata* died in those tanks that also contained *G. holbrooki*, whereas only 3% died in tanks in which *G. holbrooki* was absent. Eighty three percent of all deaths of *E. vittata* occurred in the first two weeks of the experiment, with all deaths occurring within three weeks.

Given that the tank populations of both fish species remained relatively constant over the last six weeks of the experiment, it is valid to compare fin damage of *E. vittata* to the final density of *G. holbrooki* in each tank (Fig. 1). The Model I test for linear regression indicated a highly significant linear relationship ( $p < 0.005$ ) between density of *G. holbrooki* and the fin damage in *E. vittata*, i.e., the relative fin damage in *E. vittata* was directly related to the density of *G. holbrooki* in each tank. With the exception of the 1st dorsal fin, all other fins were damaged in some individuals. However, the caudal fin was the most frequently damaged and also generally exhibited the most damage.

#### Interaction between wild populations of *Gambusia holbrooki* and native species

Linear regression showed that there was a significant inverse relationship between individual fin damage scores in *E. vittata* from the RGC system and their total lengths (at the 0.05 level of probability). The equation for this relationship is given as:

$$\text{Fin damage} = 4.67 - 0.07 (\text{TL mm})$$

Of the total of 1322 native fishes that were examined for damage to the caudal fin 219 (16.6%) showed no signs of damage, while 507 (38.4%), 333 (25.2%) and 263 (19.9%) exhibited minor, moderate and major damage, respectively (Table 1). In each species the incidence of fin damage and also the severity of that damage was higher in 0+ fish than in older fish, e.g., in *E. vittata* 14.2% of 0+ fish cf. 37.2% of older fish showed no signs of damage, whereas 23.4% of 0+ fish cf. 0.5% of older fish exhibited major fin damage. Furthermore, while the fin damage in the 0+ fish was invariably recent, damage to the fins of older fish was generally evident as scarred and/or deformed fins. Young *Bostockia porosa* appeared to be particularly susceptible to caudal fin damage; indeed the majority of 0+ *B. porosa* placed in category 3 (i.e., major damage) had little caudal fin remaining.

#### Distribution of *G. holbrooki* and *E. vittata* in rehabilitated and natural wetlands

*Edelia vittata* was captured at all sites that contained cover, irrespective of whether these sites also contained *G. holbrooki*, and also from those sites that were devoid of cover but at which *G. holbrooki* was absent (Fig. 2). In contrast, at those sites in which cover was absent but *G. holbrooki* was present *E. vittata* was never captured. The relative

Table 1. - The relative damage to the caudal fins of *Edelia vittata* (western pygmy perch), *Galaxias occidentalis* (western galaxias) and *Bostockia porosa* (nightfish) from lentic habitats in the lower south-west of Western Australia.

Species	Relative damage to caudal fin, % (n)				
	Age class (n)	0	1	2	3
<i>Edelia vittata</i>	0+ (911)	14.2 (129)	38.2 (348)	24.3 (221)	23.4 (213)
	>0+ (191)	37.2 (71)	32.5 (62)	29.8 (57)	0.5 (1)
<i>Galaxias occidentalis</i>	0+ (125)	2.4 (3)	52.0 (65)	30.4 (38)	15.2 (19)
	>0+ (23)	34.8 (8)	43.5 (10)	17.4 (4)	4.3 (1)
<i>Bostockia porosa</i>	0+ (66)	4.5 (3)	31.8 (21)	19.7 (13)	43.9 (29)
	>0+ (6)	83.3 (5)	16.7 (1)	0 (0)	0 (0)
<b>Total</b>	1322	16.6 (219)	38.4 (507)	25.2 (333)	19.9 (263)

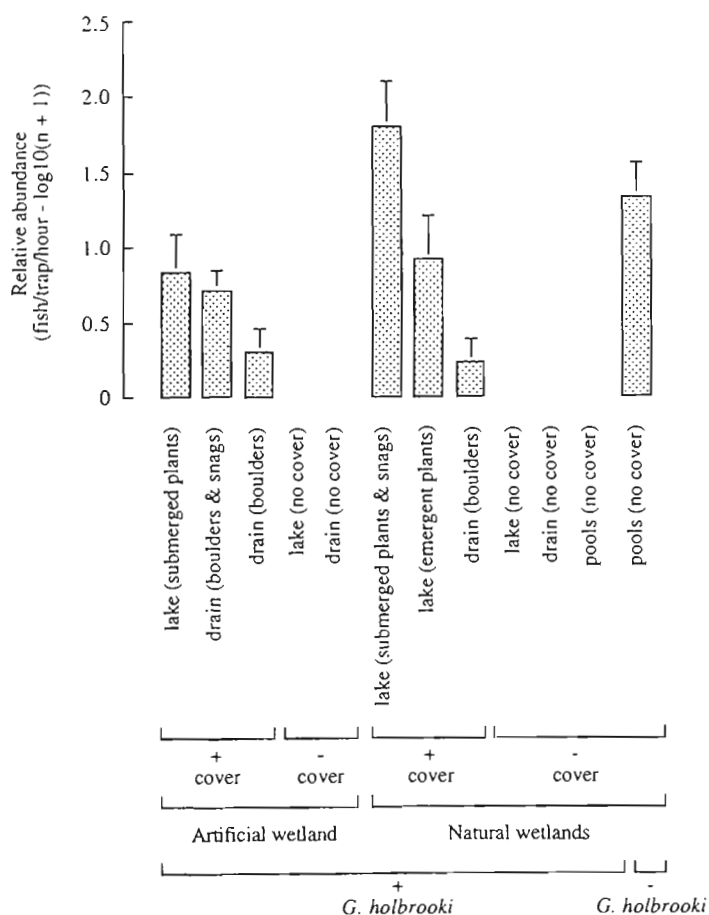


Fig. 2. - The relative abundances of *Edelia vittata* found in the different habitats within an artificial (RGC) and a natural system (Red Lake) that are infested with *Gambusia holbrooki*, and a natural system (adjacent to Red Lake) that does not contain this introduced species.

abundance of *E. vittata* (for those sites/habitats at which it was captured) ranged from a mean minimum of 1.9 fish/trap/h for the Red Lake drain sites that contained small boulders, i.e., those sites that contained only minimal cover, to a mean maximum of 141 fish/trap/h at the Red Lake sites that contained dense macrophyte growth and fallen branches, etc., i.e., those sites that contained dense and complex cover.

ANOVA indicated a highly significant difference ( $p < 0.001$ ) between the relative abundance of *E. vittata* and site/habitat type. The subsequent Scheffé test indicated that this was attributable to the higher relative abundance of *E. vittata* in those sites in Red Lake which contained submerged macrophytes and other complex cover than those sites in the drains of both Red Lake and RGC systems in which the only cover was provided by small boulders.

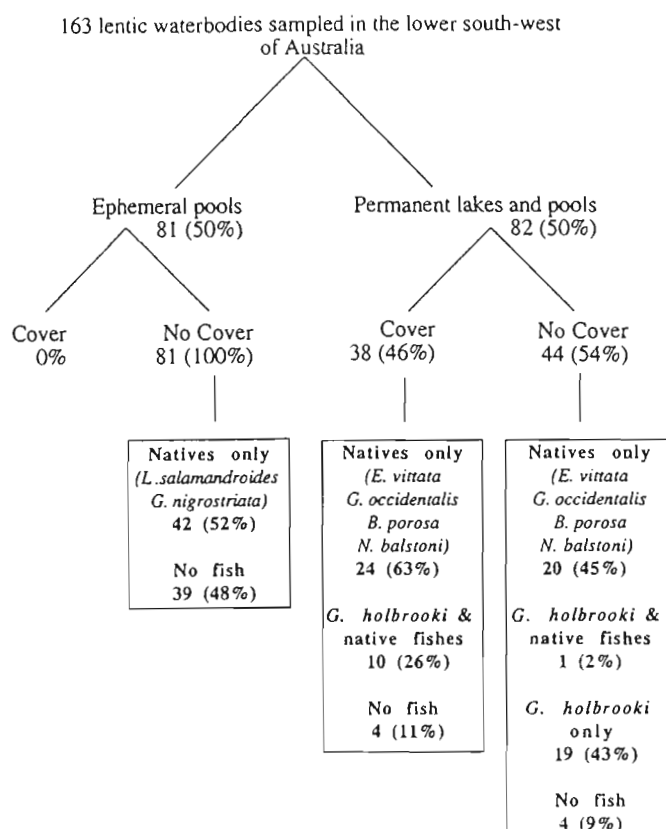


Fig. 3. - The fish fauna associated with 163 lentic waterbodies in the lower south-west of Western Australia. N.B.: While all of the native species have been recorded from each type of waterbody, for clarity, the above description records only those species that are most commonly encountered in a particular type of waterbody.

### The association between native species, *Gambusia holbrooki* and habitat type

Native species were caught in 97 of the 163 lentic waterbodies sampled (Fig. 3). Of the 82 permanent waterbodies sampled, 55 yielded native fishes while only 42 of 81 ephemeral bodies sampled contained native fishes, i.e., 67% and 52%, respectively. *Gambusia holbrooki* was only ever captured in permanent waterbodies (30 of 82, or 37%). On all but one occasion native species were only ever caught in those waterbodies that contained *G. holbrooki* when some form of cover was present. In the one instance that native fishes and *G. holbrooki* were captured in a pool that did not contain cover, only three *G. holbrooki* were caught. It should also be noted that, since this study was completed, a dense growth of aquatic plants has proliferated in a pool in the RGC system that was previously devoid of cover and native fishes, but contained large numbers of *G. holbrooki*. This pool has recently been colonised by *E. vittata*.

## DISCUSSION

### Interaction between *Gambusia holbrooki* and *Edelia vittata* in tanks

Our tank experiment demonstrated that the degree of fin damage in *E. vittata* was directly related to the density of *G. holbrooki*. Moreover, in this experiment, the vast majority of deaths of *E. vittata* occurred in those tanks that also contained *G. holbrooki*. Thus, the presence of *G. holbrooki* (in any density) affected the survival of *E. vittata*, particularly within the first two weeks of introduction. While the precise cause of death was not determined, it should be noted that the majority (~80%) of damaged fins had parasitic and/or fungal infection between the protective membranes covering the fin rays, whereas undamaged fins had little or no infection. Although damage to any fin could be severe it was generally the caudal fin that was the most severely damaged. Indeed, both in the tank experiments and in the field (all species), fish often had no caudal fin remaining.

### Interaction between wild populations of *Gambusia holbrooki* and native species and the association between native species, *G. holbrooki* and habitat type

Our results show that native species are able to co-exist with *G. holbrooki* in lentic habitats that contain cover in the form of aquatic plants, sunken timber and/or boulders. However, it also appears that these same species cannot co-exist with *G. holbrooki* in lentic waterbodies that do not contain cover.

The fact that, in the presence of *G. holbrooki*, the majority of individuals of each of the native species showed evidence of fin damage, whereas this was never the case in waterbodies in which *G. holbrooki* was absent, indicates that *G. holbrooki* exhibits the same agonistic behaviour in the wild as it does in controlled environments. It is also likely that the effect of this agonistic behaviour is similar in the wild, i.e., increased mortality rates due to fin nipping. Furthermore, the densities of *G. holbrooki* in south-south-western Australia are often at least one order of magnitude greater than those of the small native species (Hambleton *et al.*, 1996; Gill and Morgan, unpubl. data). This is likely to lead to competition for both food and space if these resources become limiting, as is the case when, for example, the small pools of the south-west contract in size during summer and autumn (Pusey and Edward, 1990). Although *G. holbrooki* has been shown to have deleterious effects on the reproductive success of *Pseudomugil signifer* (Howe *et al.*, 1997), it is unlikely that such an effect will occur in south-western Australia as the native species breed in winter to late spring, a time when *G. holbrooki* numbers are low (Pen and Potter, 1991). Likewise, this offset in the breeding period of the natives and the increase in population size in *G. holbrooki* means that piscivory on the larvae of the native fishes by *G. holbrooki* is unlikely to account for the absence of native species in small pools. However, the far greater incidence of damage to the fins of 0+ fish, and the fact that the majority of older fish showed little recent fin damage, does suggest that the juveniles of native species are particularly vulnerable to attacks by *G. holbrooki*. It would therefore appear that in waterbodies that are devoid of cover, a combination of agonistic behaviour by, and competition for both food and space with, *G. holbrooki* results in either the displacement or death of individuals of the native species. However, in those lentic waterbodies that contain both *G. holbrooki* and the native fishes, cover is likely to provide both a shelter from the agonistic behaviour of the introduced species and a substrate for some of the invertebrates on which the native fishes prey.

### **Is *Gambusia holbrooki* a threat to the native species of south-western Australia?**

This study and that of Pen and Potter (1991) indicate that while *G. holbrooki* is unlikely to lead to the extinction of populations of native fishes in either lotic systems, or lentic systems that contains cover, it is likely to affect the population size in these habitats and is potentially a threat to the populations in lentic systems that are devoid of cover. However, it must be recognised that, in south-western Australia the lentic systems that are devoid of cover are of the following types, those that are either degraded, rehabilitated or artificial and the ephemeral and permanent pools of the southern peat flats. In the case of the first three types of system, given suitable water quality, the inclusion of large amounts of cover should permit the successful recolonisation or (re)introduction of native species. However, as the vast majority of small pools in the peat flats are naturally devoid of cover for much of the year, i.e., during summer/autumn when water levels recede, it would be inappropriate to introduce snags, etc., thus changing their natural morphology. Furthermore, while only three *G. holbrooki* have been recorded from a single pool in the peat flats, this species has been present in the rivers and streams of the region for some time (Coy, 1979). It could therefore be argued that although *G. holbrooki* could colonise pools that become connected to the rivers during winters of particularly high rainfall, the chances of such colonisation must be low. Moreover, ephemeral pools can only maintain those species which can aestivate (*L. salamandroides* and *G. nigrostriata*) while the permanent pools often contain large representatives of *G. occidentalis* and *B. porosa*, both of which are piscivorous when large. Thus, unless large numbers of *G. holbrooki* are deliberately released into pools in the peat flats, we believe that this species poses little threat to the fish populations in these pools.

In conclusion, irrespective of the presumed effects of *G. holbrooki*, it is evident that this species is firmly established in a significant proportion of the waterbodies of south-western Australia, and, like most introduced species, once established is extremely difficult to eradicate. Thus, in order to encourage the spread of native fishes into rehabilitated and artificial wetlands that have been colonised by *G. holbrooki*, it is necessary to ensure that they contain adequate cover and established food webs. The simple task of the planting of emergent and submerged plants, and the inclusion of timber and other snags and also hay into artificial and degraded wetlands will not only provide cover for native fishes, but will also provide a food source for aquatic invertebrates. For example, within three weeks of hale bales being placed in newly established pools at the RGC Wetland Centre a diverse invertebrate community had become established (unpubl. data). In addition to these measures, the further spread of *G. holbrooki*, through for example deliberate releases for mosquito control or their accidental release by anglers using them for bait, must be discouraged. In this regard, local authorities (mosquito control) and anglers (bait) must be made aware of the effects, in certain habitats, that *G. holbrooki* may have on our unique and depauperate freshwater fish fauna.

**Acknowledgements.** - Financial support for this study was provided by the Water and Rivers Commission (W.A.), Fisheries Department (W.A.), National Fishcare, Natural Heritage Trust, RGC Mineral Sands and Murdoch University. Thanks are extended to Lynn, Claire and Emma Gill, and Simon Visser for their invaluable help in the field.



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## Symposium 9

### PHYLOGEOGRAPHY, GENETICS AND CHROMOSOMAL DIFFERENTIATION

Chairperson: **Catherine OZOUF-COSTAZ**

- ARNAUD S., BORSA P. & F. BONHOMME. - Mitochondrial DNA variation in the south-east Asian scad mackerel *Decapterus cf. macrosoma*. 407-411  
*Variabilité de l'ADN mitochondrial chez le chinchard Decapterus cf. macrosoma (Carangidae) en Asie du sud-est.*
- BURRIDGE C.P. - Suggestion of synonymy for *Nemadactylus* and *Acantholatris* (Perciformes: Cirrhitidae). 413-416  
*Proposition pour la mise en synonymie de Nemadactylus et Acantholatris (Perciformes: Cirrhitidae).*
- OOHARA I., SAWANO K., OKAZAKI T. & T. KOBAYASHI. - Reexamination of the molecular phylogeny of the masu salmon in the genus *Oncorhynchus*. 417-426  
*Réévaluation de la position phylogénétique du saumon masou dans le genre Oncorhynchus.*
- OZOUF-COSTAZ C., PISANO E., THAERON C. & J.-C. HUREAU. - Karyological survey of the notothenioid fish occurring in Adélie Land (Antarctica). 427-440  
*Analyse caryotypique des espèces de Notothenioidei présentes en Terre Adélie.*



## MITOCHONDRIAL DNA VARIATION IN THE SOUTH-EAST ASIAN SCAD MACKEREL *DECAPTERUS* CF. *MACROSOMA*

by

Sophie ARNAUD, Philippe BORSA & François BONHOMME (1)

Scad mackerels currently assigned to species *Decapterus macrosoma* Bleeker have a wide distribution in the tropical Indo-Pacific (Froese and Pauly, 1995). They count among the main catches by purse seiners in the South China Sea and the Java Sea (Widodo, 1988; Potier and Nurhakim, 1995). Here we report the main results of a phylogeographic survey of *D. cf. macrosoma* in the Indonesian seas. Our objective was to estimate its population genetic composition using mitochondrial (mt) DNA markers. In the process we identified three major mtDNA clades whose heterogeneous geographical distribution may point towards the occurrence of three subspecies or species in the Indonesian seas.

Samples of *Decapterus macrosoma* were collected in the South China Sea (sample size  $n = 6$ ), the Sunda Strait ( $n = 3$ ), the Java Sea ( $n = 37$ ), the Makassar strait ( $n = 51$ ), the Sulawesi sea ( $n = 46$ ), the Molucca Sea ( $n = 38$ ) and the Banda Sea ( $n = 35$ ). Total DNA was extracted from muscle tissue according to the standard phenol-chloroform extraction procedure (Sambrook *et al.*, 1989). A ca. 980-base pair fragment of the mtDNA replication control region was amplified by polymerase chain reaction (PCR) and the PCR-amplified DNA products were digested using a set of 10 restriction enzymes. Composite mtDNA haplotypes were deduced from the restriction fragment length patterns (RFLPs) that were obtained after electrophoresis of the digested DNAs.

Table I gives the geographical distribution of the 30 composite mtDNA haplotypes that were encountered in the survey. In the following we consider only those haplotypes whose frequency in a sample was  $> 0.1$ . Haplotype A was the most frequent in the samples from South China Sea, Java Sea, Makassar Strait and Sulawesi Sea. Haplotypes B and C and only these were present in the Sunda Strait sample, and only there. Haplotype H was only present in the Sulawesi Sea sample. Haplotypes P, Q, R and S were only present in the Molucca Sea and the Banda Sea samples, at frequencies that were similar. Four groups of samples were thus identified on the basis of the distribution of their most frequent haplotypes, i.e. (i) South China Sea, Java Sea, Makassar Strait (a group of samples hereafter designated by « Sunda Shelf », (ii) Sunda Strait, (iii) Sulawesi Sea and (iv) Molucca Sea and Banda Sea. No genetic differences were evident among populations from the Sunda Shelf although the occurrence of two stocks has been suspected (Sadhotomo and Potier, 1995). The Sulawesi Sea sample was characterized by the occurrence of two major mtDNA clones, one (A in Table I) that was shared with the Sunda Shelf group of samples, the other one (H) that was unique to the Sulawesi Sea sample. Actually, slight morphological variation was noted among individuals in the Sulawesi Sea sample. In short, all

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[illegible]

*macrosoma*, two of which were simultaneously present in the Sulawesi Sea. We believe that further in-depth phylogeographic studies in *D. macrosoma* and related species from the tropical Indo-Pacific will enhance our understanding of the patterns and processes of speciation in pelagic fishes.

**RÉSUMÉ. - Variabilité de l'ADN mitochondrial chez le chinchard *Decapterus cf. macrosoma* (Carangidae) en Asie du sud-est**

Le chinchard, *Decapterus macrosoma* Bleeker, est distribué dans une grande partie de l'Indo-Pacifique tropical. Il compte parmi les espèces les plus abondamment exploitées par les sennieurs en Mer de Chine du Sud et en Mer de Java. Nous rapportons ici les principaux résultats d'une étude de la structure génétique des populations de *D. cf. macrosoma* des mers indonésiennes, réalisée à l'aide de marqueurs ADN mitochondriaux (ADNmt). Nous avons identifié trois clades d'ADNmt dont la distribution géographique était hétérogène, ce qui suggère la présence de trois sous-espèces, voire espèces, dans les mers indonésiennes.

Des échantillons de *Decapterus cf. macrosoma* ont été récoltés en mer de Chine du Sud, dans le détroit de la Sonde, en mer de Java, dans le détroit de Macassar, en mer de Célèbes, en mer des Moluques et en mer de Banda. L'ADN total a été extrait à partir d'échantillons de muscle. Un fragment long d'environ 980 paires de bases de la région de contrôle de la réplication de l'ADNmt a été amplifié par la réaction cyclique de polymérisation (PCR). Les produits d'amplification par la PCR ont été digérés à l'aide de 10 enzymes de restriction. Différents haplotypes composites ont été reconstruits à partir des longueurs des fragments de restriction (RFLP) obtenues après l'électrophorèse.

Nous avons reporté dans le tableau I la distribution géographique des 30 haplotypes observés sur l'échantillon total. Considérons ici les seuls haplotypes dont la fréquence dans au moins un échantillon était  $> 0.1$ . L'haplotype A était le plus fréquent dans les échantillons de la mer de Chine du Sud, la mer de Java, le détroit de Macassar et la mer de Célèbes. Les haplotypes B et C étaient présents dans le seul échantillon du détroit de la Sonde. Les haplotypes P, Q, R et S étaient présents dans les seuls échantillons de la mer des Moluques et de la mer de Banda, à des fréquences similaires. Nous avons ainsi identifié quatre ensembles d'échantillons sur la base de la distribution des haplotypes les plus fréquents. En l'occurrence, il s'agit de (i) mer de Chine du Sud, mer de Java, détroit de Macassar (un ensemble d'échantillons que nous regrouperons sous le nom "plateau de la Sonde" dans ce qui suit), (ii) détroit de la Sonde, (iii) mer de Célèbes et (iv) mer des Moluques et mer de Banda. Aucune hétérogénéité significative n'a été détectée parmi les populations du plateau de la Sonde: ce résultat ne permet pas de confirmer l'hypothèse, découlant des données halieutiques, selon laquelle deux stocks de *D. macrosoma* seraient présents dans cette région. L'échantillon de la mer de Célèbes présentait deux haplotypes majoritaires dont le plus abondant (A) était également le plus abondant sur le plateau de la Sonde (voir Tableau I), et l'autre (H) était uniquement présent en mer de Célèbes. Signalons ici que deux variants morphologiques avaient été observés en mer de Célèbes: tous les individus présentant l'haplotype A possédaient une rangée de petites dents sur la mâchoire inférieure (ce qui est un critère de reconnaissance de *Decapterus macrosoma*). Ceux présentant l'haplotype H possédaient en plus des dents sur la mâchoire supérieure. Cette corrélation entre morphologie et haplotype mitochondrial signifie que

l'hétérogénéité de l'échantillon de la mer de Célèbes pourrait correspondre à la présence sympatrique de deux populations reproductivement isolées.

Les relations phylogénétiques entre les différents haplotypes ont été inférées à partir des données de RFLP, à l'aide d'un algorithme de parcimonie, d'une part, et d'un algorithme de maximum de vraisemblance, d'autre part, en utilisant, respectivement, les menus DNAPARS et RESTML du logiciel PHYLIP. La topologie des deux arbres ainsi inférés était la même (données non présentées ici), et celle-ci reflétait la géographie des mers indonésiennes: ainsi, tous les haplotypes (A à G) présents dans les échantillons du plateau de la Sonde et du détroit de la Sonde, en d'autres termes ceux d'Indonésie occidentale, étaient regroupés à une extrémité de l'arbre, tandis que tous les haplotypes (P-AD) présents dans les échantillons d'Indonésie orientale étaient regroupés à l'extrémité opposée de l'arbre. La plupart des haplotypes (H à O exceptés K et M) de l'échantillon de la mer de Célèbes, géographiquement intermédiaire, se positionnaient vers le milieu du segment principal. Enfin, les haplotypes K et M étaient regroupés avec l'ensemble des haplotypes "occidentaux". Les divergences nucléotidiques par paire, estimées parmi ces trois groupes d'haplotypes à l'aide du logiciel REA, étaient comprises entre 0.072 et 0.135, ce qui suggère une séparation très ancienne des populations. A titre indicatif, le taux d'évolution moléculaire moyen classiquement admis pour l'ADN mitochondrial est d'environ 2% de changement de base par million d'années.

Nous avons pu mettre en évidence une forte structure génétique au sein du taxon *Decapterus macrosoma*, ce qui indique que le flux génique entre populations est extrêmement faible. Ce résultat est inattendu chez un poisson qui effectue la totalité de son cycle biologique dans le milieu pélagique, et qui est réputé effectuer des migrations très importantes. Nos résultats démontrent également l'existence, dans les mers indonésiennes, de trois entités génétiquement distinctes - il pourrait s'agir de trois sous-espèces, voire de trois espèces - à l'intérieur de ce qui est pour l'instant considéré comme une seule espèce. Nous pensons qu'une étude approfondie de la phylogéographie du complexe « cf. *macrosoma* » dans l'Indo-Pacifique tropical nous permettra de mieux comprendre les processus conduisant à la spéciation chez les poissons pélagiques.

Key-words. - Carangidae, *Decapterus macrosoma*, ISEW, Mitochondrial DNA, Population genetics.

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## SUGGESTION OF SYNONYMY FOR *NEMADACTYLUS* AND *ACANTHOLATRIS* (PERCIFORMES: CIRRHITOIDEA)

by

Christopher P. BURRIDGE (1)

**RÉSUMÉ.** - Proposition pour la mise en synonymie de *Nemadactylus* et *Acantholatris* (Perciformes: Cirrhitidae).

Une séquence d'ADN mitochondrial du cytochrome *b* de 307 paires de bases a été obtenue et analysée pour cinq espèces du genre *Nemadactylus* et deux espèces du genre *Acantholatris*. Les résultats suggèrent que ces deux genres sont synonymes. Cette proposition est également supportée par la découverte récente d'une nouvelle espèce de *Nemadactylus*, le "king tarakihi", qui invalide la distinction entre ces deux genres sur la base de caractères morphologiques.

Key-words. - Cirrhitidae, *Nemadactylus*, *Acantholatris*, Mitochondrial DNA, Synonymy.

At present there are five recognised species of *Nemadactylus*. Four of these are from the waters of Australia and New Zealand, and the remaining species occurs along the east coast of South America (Table I). One of these species has only been proposed recently (Roberts, 1993; Smith *et al.*, 1996). Yet to be named, it is commonly called "king tarakihi", either in reference to its greater size than the "tarakihi", *N. macropterus*, or its capture from the Three Kings Islands region in New Zealand (Smith *et al.*, 1996).

In contrast to *Nemadactylus*, the genus *Acantholatris* is not represented in the waters of Australia or New Zealand. Instead, the three species of *Acantholatris* occur around isolated islands and seamounts such as Juan Fernández in the southeastern Pacific, and those which form a loose chain from Tristan da Cunha and Gough Island in the South Atlantic, to Saint Paul and Amsterdam Islands in the Indian Ocean (Table I).

*Nemadactylus* and *Acantholatris* have been separated primarily on the number of anal fin rays (Gill, 1862), with the former having 14-19, and the latter 10-12 (Lamb, 1990). Under this definition the South American species *bergi*, with 14-15 anal fin rays, does not belong in *Acantholatris* as listed by Greenwood (1995). This species is also commonly referred to as a *Cheilodactylus* in South American literature, but it is obviously a member of *Nemadactylus* and is very similar to *N. macropterus* (Lamb, 1990; Esteban, pers. comm., Centro Nacional Patagónico, Puerto Madryn, Argentina).

As part of a wider study on the molecular phylogeny of cirrhitoid fishes, 307 base pair mitochondrial cytochrome *b* DNA sequences have been obtained from all five species of *Nemadactylus*, and two of the three species of *Acantholatris*: *A. monodactylus* and *A. gayi*. Analysis of these sequences by a number of methods, with the inclusion of data from other cirrhitoid genera, consistently resulted in *A. monodactylus* and *A. gayi* clustering within a well supported and otherwise entirely *Nemadactylus* clade. In addition, the level of sequence variation between these two species of *Acantholatris* with *Nemadac-*

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*tylus* sp. (king tarakihi), *N. macropterus*, and *N. bergi* was minimal. These results suggest that *Nemadactylus* and *Acantholatris* are synonymous. If supported by other evidence *Nemadactylus* would have priority.

Although there were no molecular data for *A. vemae* in these analyses, it is most likely that this species would also be closely related to the other species of *Acantholatris*, and group within the *Nemadactylus* clade. *Acantholatris vemae* is very similar in morphology to the other species of *Acantholatris* (Lamb, 1990), and occurs in sympatry with *A. monodactylus* at Vema Seamount in the South Atlantic Ocean (Penrith, 1967).

The suggestion of generic synonymy on the basis of molecular data is also supported by the recently identified king tarakihi species, which falsifies the present morphological distinction of *Nemadactylus* and *Acantholatris*. The king tarakihi is similar in appearance to *N. macropterus*, and they occur sympatrically (Smith *et al.*, 1996). However, this new species possesses only 12 anal fin rays, not 14 or more. Therefore, despite its similarity in appearance and habitat with *N. macropterus*, the king tarakihi would have to be described as *Acantholatris* under current generic definition.

Although Roberts (1993) and Smith *et al.* (1996) have distinguished the king tarakihi from *N. macropterus* with both morphological and molecular characters, the anal fin ray counts from this new species are identical to *A. gayi* from Juan Fernández. Although few other characters are available for comparison, the king tarakihi may only represent a new record of *A. gayi* in the waters of Australia and New Zealand, and not a new species as suggested on the basis of comparison against *N. macropterus* alone.

A taxonomic revision by Greenwood (1995) based on urohyal morphology suggested that both *Nemadactylus* and *Acantholatris* belong within the Latridae, and not the Cheilodactylidae as currently understood. While the molecular data obtained to date do not support such a transfer (Burrige, unpubl. data), the familial allocation of these species has no influence on the outcome of this work.

It is proposed that the five most closely related species of *Nemadactylus* and *Acantholatris* have radiated and dispersed widely throughout the Southern Hemisphere in recent times, conservatively within the last c 0.5 million years when using a molecular clock calibration of 2.3% sequence divergence per million years (Martin *et al.*, 1992). Such recent and long distance dispersal could have been mediated by the 9-12 month pelagic larval period observed in this group (Vooren, 1972; Andrew and Hecht, 1992). High dispersal capabilities have also been implicated for the lack of genetic differentiation among

Table I. - Species of *Nemadactylus* and *Acantholatris*.

Species	Distribution
<i>Nemadactylus valenciennesi</i>	southern Australia
<i>Nemadactylus douglasii</i>	southeastern Australia, northern New Zealand
<i>Nemadactylus macropterus</i>	southern Australia, New Zealand
<i>Nemadactylus</i> sp. ("king tarakihi")	eastern Australia, northern New Zealand
<i>Nemadactylus bergi</i>	Argentina, Uruguay
<i>Acantholatris monodactylus</i>	Tristan/Gough/Vema to St. Paul/Amsterdam Islands
<i>Acantholatris vemae</i>	Vema Seamount
<i>Acantholatris gayi</i>	Juan Fernández

*N. macropterus* populations in the waters of southern Australia (Elliott and Ward, 1994; Grewe *et al.*, 1994).

As the cytochrome *b* sequences do not reveal enough variation among the five most closely related species of *Nemadactylus* and *Acantholatris* to resolve their exact relationships, the possible directions of this dispersal are under investigation using more variable DNA sequence data. Both the West Wind Drift current (Eschmeyer and Hureau, 1971; Andrew *et al.*, 1995) and the northern components of anticyclonic gyres (Collette and Parin, 1991) may have been involved.

Questions of specific status and population genetics involving several of the most closely related species of *Nemadactylus* and *Acantholatris* are currently being addressed with microsatellites.

**Acknowledgements.** - The following people assisted with the provision of fish tissues for this study: German Pequeño, Universidad Austral de Chile; Guillermo Canete, INIDEP, Argentina; Guy Duhamel, MNHN, France; Leslie Ter Morshuizen, J.L.B. Smith Institute, South Africa; Malcolm Francis, NIWA, Wellington, New Zealand; Mark McGrouther, Australian Museum; Patrick Coutin, MAFRI, Victoria, Australia; Robert Rigoni, South Australia; Tim Andrew, Rhodes University, South Africa. Robert White provided constructive criticism of an early draft of the manuscript.

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## REEXAMINATION OF THE MOLECULAR PHYLOGENY OF THE MASU SALMON IN THE GENUS *ONCORHYNCHUS*

by

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**ABSTRACT.** - In order to gain insight into the phylogenetic relation of the masu salmon, *Oncorhynchus masou masou*, in the genus *Oncorhynchus*, we reanalyzed 2,162 base pair sequences from the mitochondrial genome of the eight Pacific salmonid species and of the Atlantic salmon *Salmo salar*. These DNA sequences span from the 3' region of the gene for ATPase subunit 6 to the 5' region of the gene for NADH dehydrogenase subunit 4L. The evolutionary distances among the nine species were calculated according to the Tamura-Nei method for the third codon positions of the protein coding regions. These distances were then used to construct a neighbor-joining tree. Further, a maximum likelihood tree was constructed. The evolutionary trees that were obtained suggest that the masu salmon first diverged from the common ancestor of the genus *Oncorhynchus*, though the bootstrap probabilities supporting this are not high. As both of the two methods used for tree constructions incorporate the effects of the strongly biased frequencies of the four nucleotides in salmonid mitochondrial DNAs, the obtained trees are more reliable than those previously reported on the bases of Kimura 2-parameter model and maximum parsimony analyses.

**RÉSUMÉ.** - Réévaluation de la position phylogénétique du saumon masou dans le genre *Oncorhynchus*.

Pour repréciser la position phylogénétique du saumon masou dans le genre *Oncorhynchus*, une séquence d'ADN mitochondrial de 2162 paires de bases a été analysée pour 8 espèces de Salmonidae du Pacifique et pour le saumon atlantique *Salmo salar*. Ces séquences sont comprises entre la région 3' du gène pour la sous-unité 6 de l'ATPase et la région 5' du gène pour la sous-unité 4L de la NADH deshydrogénase. Les distances génétiques entre ces neuf espèces ont été calculées par la méthode de Tamura-Nei pour les positions du troisième codon des régions codantes. Deux arbres phylogénétiques ont été obtenus par la méthode du neighbor-joining et du maximum de vraisemblance. Les phylogénies obtenues suggèrent que le saumon masou aurait été le premier à diverger d'un ancêtre commun au genre *Oncorhynchus* bien que cette branche ne soit pas supportée par une valeur de bootstrap très forte. Les deux méthodes de construction d'arbres utilisées ici tenant compte du fort biais de composition nucléotidique observé dans les gènes mitochondriaux des salmonidés, les arbres obtenus sont plus fiables que ceux qui ont été précédemment publiés sur la base du modèle de Kimura à deux paramètres ou utilisant une méthode de parcimonie.

**Key-words.** - Salmonidae, *Oncorhynchus*, *Oncorhynchus masou masou*, Evolution, Mitochondrial DNA, MEGA, PHYLIP, Protein coding sequence, F84 model, Tamura-Nei model, Maximum likelihood.

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The masu salmon *Oncorhynchus masou masou* has intrigued many biologists for years with its phylogenetic relation to other salmonids. Its distribution pattern is rather unique in the genus *Oncorhynchus*, since it only occurs along the western side of the North Pacific (Masuda *et al.*, 1984). The masu salmon shares several primitive morphological and behavioral characters with the rainbow trout *Oncorhynchus mykiss* (Neave, 1958).

Since Neave's (1958) hypothesis on the origin of salmonids, the evolutionary relationships among salmonid fish including the masu salmon have been examined extensively (Smith and Stearley, 1989) using morphology and ecology (Hikita, 1962; Gorshkov *et al.*, 1979), physiology and life history (Hoar, 1976) and protein electrophoretic variability (Tsuyuki and Roberts, 1966; Yoshiyasu, 1973; Utter *et al.*, 1973). According to the hypothesis proposed by Neave (1958), which agreed well with the morphologically and ecologically obtained tree (Smith and Stearley, 1989), the *Salmo*-like progenitor gave rise to two monophyletic groups: one evolutionary line including the rainbow trout and the cutthroat trout, and another one including the masu salmon among other *Oncorhynchus* species.

On the other hand, studies on hybridization experiments between the masu salmon and other salmonids (reviewed by Chevassus, 1979) as well as on the hemoglobin electrophoretic variability (Yoshiyasu, 1973) and allozymes (Utter *et al.*, 1973) emphasized the similarity between the masu salmon and the rainbow trout.

New methodologies for phylogenetic studies have appeared, based on mitochondrial (mt) DNA analysis (Lansman *et al.*, 1981; Brown *et al.*, 1982; Berg and Ferris, 1984; Thomas *et al.*, 1986). Comparisons were made for a 2,214 base pair sequence of mtDNA among six Pacific *Oncorhynchus* species (Thomas and Beckenbach, 1989). They proposed a new phylogenetic tree by analyzing the nucleotide divergence observed in pairwise comparisons among the species. Unfortunately, they omitted the masu salmon from their sequence analysis of mtDNA.

Subsequently, Shedlock *et al.* (1992) analyzed the mitochondrial control region sequences of a variety of salmonids, including the masu salmon. According to their phylogenetic tree, the masu salmon appeared after the divergence of the rainbow trout and cutthroat trout line, and the line of coho salmon and chinook salmon. However, it is quite interesting that, based on the same DNA sequences with Shedlock *et al.* (1992) but with more reliable mathematical methods, Saitou and coworkers (Kitano *et al.*, 1997) suggested that masu salmon diverged first in the Pacific salmon species.

A totally different molecular biological approach was taken by Okada and coworkers (Murata *et al.*, 1996): the insertions of SINEs (short interspersed elements) into the salmonid genomes were used for determining their branching orders. Unfortunately again, they have not yet succeeded in finding a SINE which should clarify the branching order of masu salmon among the species in *Oncorhynchus*.

Our previous study (Oohara *et al.*, 1997) examined the phylogenetic relations of the masu salmon in the genus *Oncorhynchus* based on the sequence of their mtDNA, mainly the protein coding regions (ATPase subunit 6, cytochrome oxidase subunit 3, NADH dehydrogenase subunit 3 and NADH dehydrogenase subunit 4L). The obtained trees suggested that the masu salmon first diverged from the common ancestor of the genus *Oncorhynchus*. These analyses, however, did not take into account the strongly biased frequencies of the four nucleotides at the third codon positions of the protein coding regions of salmonid mtDNAs. Furthermore, the use of the maximum parsimony analyses was a little problematic for the data set in our previous study, because (1) the multiple hits



were not precisely corrected, especially for the branch to the outgroup, and (2) there was no mathematical rationale to give a certain weight to a transversion versus a transition.

Our aim in this work is to obtain improved phylogenetic trees of the genus *Oncorhynchus* including the masu salmon, with mathematically high reliabilities. For this purpose, the distance method based on Tamura-Nei (1993) model combined with the neighbor-joining (NJ) method (Saitou and Nei, 1987) has been used, instead of Kimura (1980) two-parameter method used in the previous study. The maximum likelihood method (Felsenstein, 1993) based on F84 model (Kishino and Hasegawa, 1989) has also been adopted instead of maximum parsimony method.

## MATERIALS AND METHODS

The sources of the mtDNA sequences used in this study are indicated in our previous paper (Oohara *et al.*, 1997). The DNA sequences determined by our group have been deposited in the DDBJ/GenBank/EMBL Data Libraries, under Accession Nos. D63336 for masu salmon *Oncorhynchus masou masou*; D84147 for chum salmon *O. keta*; and D84148 for Atlantic salmon *Salmo salar*. The sequences for six other Pacific salmonids were taken from Thomas and Beckenbach (1989): rainbow trout *Oncorhynchus mykiss*; cutthroat trout *O. clarki*; chinook salmon *O. tshawytscha*; coho salmon *O. kisutch*; sockeye salmon *O. nerka*; and pink salmon *O. gorbuscha*.

DNA sequence data were handled with Genetyx software programs (Software Development Co Ltd, Japan). The MEGA Version 1.01 computer program (Kumar *et al.*, 1993) was used for Tamura-Nei (1993) distance and NJ method (Saitou and Nei, 1987). The program DNAML in PHYLIP 3.5c computer package (Felsenstein, 1993) was used for maximum likelihood analysis. The programs SEQBOOT and CONSENSE in PHYLIP 3.5c were used for bootstrap analysis.

## RESULTS

### Choice of the sites for the evolutionary analyses

The mtDNA region used for the analyses covers the 3' portion of the structural gene of ATPase subunit 6 (ATPase 6), the gene for cytochrome oxidase subunit 3 (CO III), the glycine tRNA gene, the gene for NADH dehydrogenase subunit 3 (ND3), the arginine tRNA gene, and the 5' portion of the gene for NADH dehydrogenase subunit 4L (ND4L).

In order to get information on the phylogenetic relationships between the masu salmon and other salmonids, only the third codon positions of the protein coding sequences were used. The tRNA genes and the first and second codon positions of the protein coding regions were omitted from the analyses, because their evolutionary constraints are completely different from the third codon positions. In total, 663 third codon positions were compared among the nine salmonid species.

### Examination of the base frequency constancy among the compared species

The two molecular evolutionary models which we dealt with are Tamura-Nei model (Tamura and Nei, 1993; Kumar *et al.* 1993) for the distance method, and Felsenstein F84 model described in equation (3) in Kishino and Hasegawa (1989) for the maximum likeli-

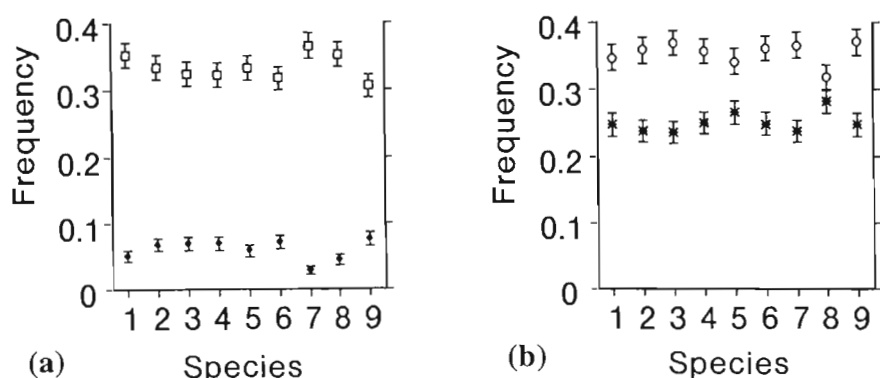


Fig. 1. - The four base frequencies at the 663 third codon positions of the protein coding regions for nine salmonid species examined. The marks for the bases are: (a), open box, adenine A; filled circle, guanine G; (b), open circle, cytosine C; star, thymine T. The species are: 1, chinook salmon *Oncorhynchus tshawytscha*; 2, coho salmon *O. kisutch*; 3, rainbow trout *O. mykiss*; 4, sockeye salmon *O. nerka*; 5, cutthroat trout *O. clarki*; 6, pink salmon *O. gorbuscha*; 7, Atlantic salmon *Salmo salar*; 8, masu salmon *Oncorhynchus masou masou*; and 9, chum salmon *O. keta*. Each point is accompanied with the corresponding standard deviation calculated as described in the legend of table I.

hood method. As both of the two models are based on the assumption of Markov process, the frequencies of the four bases should be conserved among the species compared. The examination of this criterion was done as described in Lanave *et al.* (1984). The results are given in figure 1 and table I.

Table I. - Test of the four base frequency conservation at the 663 third codon positions examined among the nine salmonid species. a) This standard deviation is a theoretical one calculated with the assumption of multinomial distribution, as:  $\sigma = \sqrt{P_A(1 - P_A)/N}$ , where  $N = 663$ , and in this case,  $P_A = 0.353$ . b) This standard deviation is an empirical one calculated from the nine values listed above, i.e.,  $\sigma_{n-1}$ , where  $n = 9$  (number of species). c) This  $\chi^2$  value shows that the frequency of guanine base is not conserved among the nine species, because the range of  $\chi^2$  values with degree of freedom of 8 is from 2.18 to 17.53 ( $p > 0.05$ ).

Species	Base frequencies (%)			
	Adenine	Guanine	Cytosine	Thymine
chinook	35.3±1.9 a)	5.13±0.86	34.8±1.9	24.7±1.7
coho	33.5±1.8	6.79±0.98	35.9±1.9	23.8±1.7
rainbow	32.6±1.8	6.94±0.99	37.0±1.9	23.5±1.7
sockeye	32.3±1.8	6.94±0.99	35.8±1.9	25.0±1.7
cutthroat	33.3±1.8	5.88±0.91	34.2±1.8	26.6±1.7
pink	31.8±1.8	7.09±1.00	36.2±1.9	24.9±1.7
Atlantic	36.7±1.9	2.87±0.65	36.7±1.9	23.8±1.7
masu	35.3±1.9	4.52±0.81	32.0±1.8	28.2±1.8
chum	30.6±1.8	7.54±1.03	37.1±1.9	24.7±1.7
Average	33.5±1.9 b)	5.97±1.52	35.5±1.6	25.0±1.5
$\chi^2$	8.81	33.08 c)	6.29	6.05



animal mtDNAs (Brown, 1985; Wilson *et al.*, 1985) including fish (Thomas and Beckenbach, 1989). The evolutionary distance of each pairwise combination of two sequences was calculated (Table II) using Tamura-Nei (1993) method, which can incorporate both the inequality of transition rate versus transversion rate and the base frequency biases.

Figure 2 shows the evolutionary tree drawn by the NJ method (Saitou and Nei, 1987), which is based on the corrected evolutionary distance in table II. The Atlantic salmon *Salmo salar* was used as an outgroup for the genus *Oncorhynchus*. The 50% majority rule consensus tree was also obtained by bootstrap analysis with 1,000 replications, whose topology coincided with that in figure 2. According to the tree, the 8 species in *Oncorhynchus* are divided into four groups, namely: masu salmon (1); the group of coho and chinook salmon (2); rainbow and cutthroat trout (3); sockeye, pink and chum salmon (4). According to the tree, the masu salmon diverged first from the common ancestor of the genus *Oncorhynchus*.

#### Analysis using the maximum likelihood method

The maximum likelihood method (Kishino and Hasegawa, 1989; Felsenstein, 1993), another algorithm for obtaining the phylogenetic tree, was also employed. The current version of the PHYLIP 3.572 (can be easily downloaded from Internet) contains a program called DNAML, which allows us the maximum likelihood estimation of the phylogenetic trees. The program is based on the model F84 by Felsenstein, being described in Kishino and Hasegawa (1989). The value of the transition/transversion ratio should be determined by the users. The default value of the ratio is set to 2.0, which is equivalent to  $\alpha/\beta = 4.0$  in Kimura (1980) 2-parameter model, in the case of equal frequencies of the four bases. In order to determine the best value of the transition/transversion ratio, DNAML was repeatedly used with many different values of this variable.

Figure 3 shows the obtained values of Ln Likelihood plotted against the transition/transversion ratio. The value of maximum likelihood was obtained with transition/transversion ratio  $\alpha/\beta = 6.172$ .

Figure 4 presents the maximum likelihood tree obtained, with the transition/transversion ratio being 6.172. The topology of the tree, however, was identical for a wide range of transition/transversion ratio, from 1.0 to 15.0. The maximum likelihood tree topology is the same with that obtained by the distance method (Fig. 2). A 50% majority rule consensus tree was obtained by bootstrap analysis with 1,000 replications. The consensus tree topology also coincided with that in figure 4. Similar scores of bootstrap probability with those in figure 2 were obtained. Although figure 4 again suggests that the masu salmon first diverged from the ancestor of the genus *Oncorhynchus*, the bootstrap probability supporting this idea is low (31%).

## DISCUSSION

We have reexamined the mtDNA sequence data of salmonids in the light of mathematical rigor. Because the average frequency of the guanine base is only about 6.0% (Table I), being strongly biased from the expected value (25%) in the case of the equal frequencies for all of the four bases, it is necessary to incorporate the distorted base frequencies into the analyses for obtaining reliable trees. Also important is the incorporation of the high transition/transversion ratios observed in mtDNAs. There are at least four mathematical models which fulfill the two criteria mentioned above: General time-

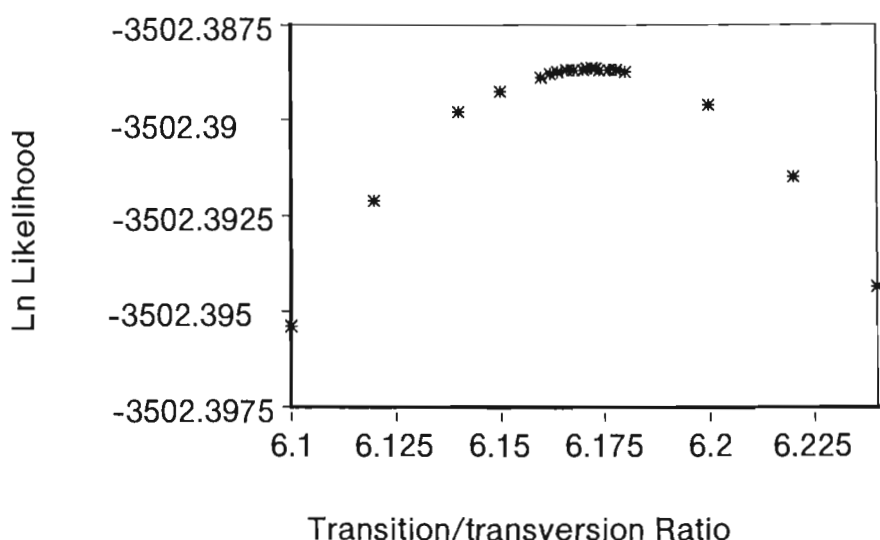


Fig. 3. - Value of Ln Likelihood plotted versus the transition/transversion ratio. Each star corresponds to one analysis by DNAML (Felsenstein, 1993) with one transition/transversion ratio. The maximum likelihood (Ln Likelihood = -3502.38864) was obtained when the transition/transversion ratio was 6.172.

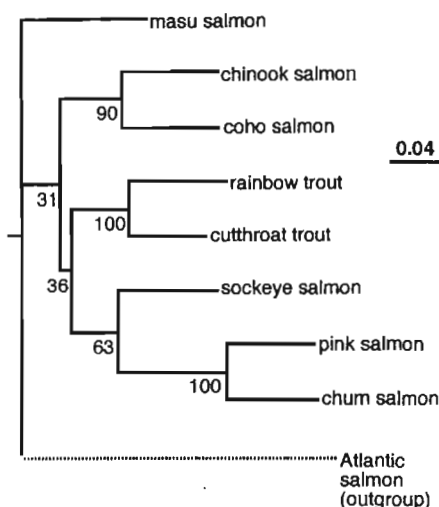


Fig. 4. - Maximum likelihood tree for the nine salmonids. The program DNAML (Felsenstein, 1993) with F84 model by Felsenstein (Kishino and Hasegawa, 1989) was used. The bootstrap probability (%) with 1,000 replications is shown for each cluster.

reversible model (Lanave *et al.*, 1984); Felsenstein's F84 model (Kishino and Hasegawa, 1989); HKY model (Hasegawa *et al.*, 1985); and Tamura-Nei (1993) model. The distance method we have adopted here (Table II; Fig. 2) uses the fourth model, while the maximum likelihood method in the program DNAML used for figures 3 and 4 corresponds to the second model. This F84 model is very similar but not perfectly identical to the HKY model. We did not use the first model by Lanave *et al.* (1984) because the model contains too many parameters.

It is thus quite interesting and educative to compare the resulted trees in this work with those in our previous report (Oohara *et al.*, 1997) which are based on simpler mathematical formulae or hypotheses. The main advance in this series of analyses from the former report is the incorporation of the unequal usage of the four bases (Table I).

The two trees obtained in this work (Figs 2, 4) perfectly agree not only with each other but also with the previously obtained trees (Oohara *et al.*, 1997) with respect to the topology. Lower bootstrap probabilities, however, are obtained in this work than in the previous study. Interestingly, for nearly all clusters, lower bootstrap probabilities are obtained with the transition/transversion ratio giving the maximum likelihood than that of default (2.0). This means that the most likelihood estimate does not necessarily give highest bootstrap probabilities, though it should give more reliable bootstrap probabilities than estimated by the default.

Our evolutionary trees suggest that four phylogenetic groups (masu salmon (1), the group of coho and chinook salmon (2), rainbow and cutthroat trout (3), sockeye, pink and chum salmon (4)) are likely to have diverged within a relatively short period of time. This idea is consistent with the maximum likelihood tree of Shedlock *et al.* (1992), and with the NJ tree by Kitano *et al.* (1997).

Our results suggest the possibility that the masu salmon first diverged from the common ancestor of the genus *Oncorhynchus*. However, the bootstrap probability supporting this idea is 59% in figure 2 and 31% in figure 4, being not high enough to conclude so. Longer mtDNA sequence data and/or the use of nuclear markers would be necessary to increase the reliability of this suggestion.

The origin of apparent disagreement between our tree and that of Shedlock *et al.* (1992) is not clear. They analyzed one kilo base sequence of the mitochondrial control or D-loop region. They also chose Atlantic salmon as an outgroup for constructing their tree. The number of polymorphisms scored is larger in our study than Shedlock *et al.* (1992), supporting the statistical reliableness of our data. Because the control region contains not only sequences highly variable of unknown importance but also many stretches of conservative sequences, the polymorphisms in the control region may not always be considered as evolutionarily neutral. On the other hand, the base substitutions in the third codon positions studied in this paper can be regarded as almost perfectly neutral, because most of the substitutions were synonymous, which do not change the amino acid coded.

Another origin of difference between the two studies may be that there are essentially no insertions or deletions observed in our sequences from protein coding regions, whereas many more insertions/deletions were found in Shedlock *et al.* (1992): in their analysis, gaps were treated as the 5th character states in addition to the four nucleotide states. Our analyses do not contain any arbitrary assumptions such as parameters concerned in the change between the deleted state and the state-A, T, G or C. It should also be noted that Kitano *et al.* (1997) eliminated gaps from the realigned sequences of the control regions obtained by Shedlock *et al.* (1992) and obtained results more consistent with ours.

Our suggestion that the masu salmon may have diverged first from the common ancestor of the genus *Oncorhynchus* is not consistent with Neave's (1958) hypothesis. According to Neave, the rainbow trout should be an outgroup of the rest of the genus *Oncorhynchus*. On the other hand, our data suggest that masu salmon is the most primitive type in *Oncorhynchus*. Neave's (1958) hypothesis may be biased by the idea that the rainbow trout should be a species in *Salmo*, which is not true now (Smith and Stearley,

1989). From these points of view, Neave's (1958) hypothesis should be critically reexamined, and at the same time, the efforts to obtain more and more reliable views on the phylogenetic association of the masu salmon to the genus *Oncorhynchus* will be necessary, on the basis of molecular evolutionary genetics.

**Acknowledgements.** - We thank Drs Kazumi Hosoya, Nobuhiro Minaka and Koichiro Tamura for stimulating discussions.

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## KARYOLOGICAL SURVEY OF THE NOTOTHENIOID FISH OCCURRING IN ADELIE LAND (ANTARCTICA)

by

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Christelle THAERON (1) & Jean-Claude HUREAU (1)

**ABSTRACT.** - One channichthyid (*Chionodraco hamatus*) and six nototheniids (*Notothenia coriiceps*, *Trematomus hansonii*, *T. pennellii*, *T. bernacchii*, *T. newnesi*, *T. loennbergi*) have been studied karyologically using conventional Giemsa-, silver-, CMA<sub>3</sub>-stainings and C-banding. The comparisons of the results from these Adelie Land specimens with those already obtained in other Antarctic sectors for the same species show substantial intra-specific variation at both chromosomal macro- and microstructure levels, and also variability in occurrence of differentiated sex chromosomes. The rearrangements producing those diverse karyomorphs may have an adaptative character and could be used for identification of and comparison between populations.

**RÉSUMÉ.** - Analyse caryotypique des espèces de Notothenioidei présentes en Terre Adélie.

L'analyse chromosomique d'un Channichthyidae (*Chionodraco hamatus*) et de six Nototheniidae (*Notothenia coriiceps*, *Trematomus hansonii*, *T. pennellii*, *T. bernacchii*, *T. newnesi*, *T. loennbergi*) a été réalisée en Terre Adélie en utilisant les méthodes de coloration au Giemsa, au nitrate d'argent et à la CMA<sub>3</sub> ainsi que le marquage des bandes C. La comparaison avec les résultats obtenus sur les mêmes espèces dans d'autres secteurs de l'Antarctique révèle une importante variabilité intraspécifique au niveau de la micro- et de la macrostructure chromosomiques, ainsi que dans la présence de chromosomes sexuels différenciés. Il est probable que les réarrangements produisant ces différents caryomorphes sont de type adaptatif et que ce critère pourrait être utilisé pour la comparaison et l'identification des populations.

**Key-words.** - Notothenioidei, PSE, Antarctica, Adelie Land, Chromosomes, Intra-specific polymorphism.

In January-February 1996, the "ICOTAÓ programme was carried out in Adelie Land by a multidisciplinary Franco-Italian team. The aims were to conduct an exhaustive assessment of the fish fauna between depths of 0 to 400 m, including systematics (morphology, ontogeny, karyology, biochemistry and molecular biology) and general biology. Seven species were kept alive and thus studied karyologically. All belong to the suborder Notothenioidei which is endemic of the Southern Ocean and comprises at least 8 families. We present here the cytogenetic study of one channichthyid, *Chionodraco hamatus* and six nototheniids (*Notothenia coriiceps*, *Trematomus hansonii*, *T. pennellii*, *T. bernacchii*, *T. newnesi*, *T. loennbergi*) using conventional Giemsa staining and several

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banding techniques: silver staining of nucleolar organizing regions (NORs), Chromomycin A<sub>3</sub> (CMA<sub>3</sub>) fluorescent staining and C-banding, which reveal constitutive heterochromatin blocks. Herein we focus on the comparison with results previously obtained on the same species in other Antarctic regions and on the role chromosomes may have played in speciation.

## MATERIALS AND METHODS

Throughout the paper, we use the taxonomy for Nototheniidae species and genera established by DeWitt *et al.* (1990).

The following species have been analysed (for each of them, several reference specimens have been deposited in the Muséum national d'histoire naturelle, Paris (MNHN). Their registration numbers are indicated below):

*Chionodraco hamatus* (Lönnberg, 1905). 15 males, 6 females; MNHN 1996-0317;

*Notothenia* (*Notothenia*) *coriiceps* Richardson, 1844. 14 males, 8 females; MNHN 1996-0357/0358;

*Trematomus pennellii* Regan, 1914. 2 females; MNHN 1996-0320/0321;

*Trematomus hansonii* Boulenger, 1902. 3 males, 7 females; MNHN 1996-0308/0309;

*Trematomus newnesi* Boulenger, 1902. 1 female; MNHN 1996-0328;

*Trematomus bernacchii* Boulenger, 1902. 7 males, 23 females; MNHN 1996-0334 to 0343;

*Trematomus loennbergi* Regan, 1913. 2 females; MNHN 1996-0325/0326.

Chromosome preparations were made in the field from cephalic kidney and spleen cell suspensions of colchicized animals (Doussau de Bazignan and Ozouf-Costaz, 1987) and fixed suspensions were deep-frozen. Giemsa conventional staining (Hartley and Horne, 1985), silver-staining (Howell and Black, 1980), CMA<sub>3</sub> fluorescent staining (Schweizer, 1976) and C-banding (Sumner, 1972) were made later in our laboratories.

The following abbreviations will be used in the text and table I: 2n: diploid chromosome number; m: metacentric chromosome; sm: submetacentric chromosome; st: subtelocentric chromosome, i.e. biarmed, with a visible very short arm; a: acrocentric chromosome, this category includes one-arm chromosome plus st chromosomes; NF: arm number (also called fundamental number); NORs: nucleolar organizing regions; Ag-NORs: silver positively stained NORs; CMA<sub>3</sub>: chromomycin A<sub>3</sub>.

## RESULTS AND DISCUSSION








There are important differences between some of our results and those obtained for the same species in other sectors of the Antarctic Ocean. To facilitate understanding, the available data for each species are summarized in table I by geographic sector, including publication references, which will thus not be cited in the following discussion.

### Channichthyidae

#### *Chionodraco hamatus*

Diploid chromosome number is  $2n = 47/48$  ( $2m + 4sm + 41/42a$ ). As in other Antarctic sectors this icefish possesses differentiated multiple sex chromosomes

Table I. - Karyotypes of the seven species studied in Adelle Land and other karyological data obtained for the same species in other Antarctic regions. N: number of specimens analysed; undet: sex not determined or immature; m: male; f: female; NF: arm number; REF.: (1): present work; (2): Morescalchi *et al.*, 1992a; (3): Morescalchi *et al.*, 1992b; (4): Ozouf-Costaz *et al.* 1991; (5): Prirodina and Neyelov, 1984; (6): Phan *et al.*, 1986; (7): Ozouf-Costaz, unpubl. data.

SPECIES	LOCALITY	undet	N m	f	undet	2n m	f	FORMULA	NF	Ag-NORs	Ag-NOR number	C-banding	CMA3	REF.
<b>CHANNICHTHYIDAE</b> <i>Chionodraco hamatus</i> 	Adelle Land Ross Sea Weddell Sea	- - -	15 >3 -	6 >3 2	- - -	47 47 48	48 48 48	2m+4sm+42a " "	54 54 54	+ + +	2 2 2	+ + -	+ + -	1 2 4
<b>NOTOTHENIIDAE</b> <i>Notothenia coriiceps</i> 	Adelle Land Ross Sea Arctowski (Bransfield Strait) Bransfield strait Signy Island	- - - 1 -	14 1 - - 3	8 - 5 - 3	- - - 22 -	22 22 - - 22	22 - 22 - 22	16m+6sm 18m+4sm 18m+2sm+2a 20m+2sm 20m+2sm	44 44 42 44 44	+ + - - -	2 2 - - -	+ + - - -	+ - - - -	1 3 5 7 8
<i>Trematomus pennellii</i> 	Adelle Land Ross Sea Weddell Sea	- - -	- 4 1	2 3 1	- - -	- 32 32	32 32 32	14m-sm+18a 14m-sm+18a 14m-sm+18a	46 46 46	+ + -	1 or >1 2 -	+ - -	+ - -	1 3 4
<i>Trematomus hansonii</i> 	Adelle Land Ross Sea Weddell Sea Bransfield Strait	- - - 2	3 >3 - -	7 >10 1 -	- - - 48	46 46 48 -	46 46 48 -	4m+2sm+40a 4m+2sm+40a 2m+4sm+42a 2m+4sm+42a	52 52 54 54	+ + + -	44 2 2 or 3 -	+ - - -	+ - - -	1 3 4 6
<i>Trematomus newnesi</i> 	Adelle Land Ross Sea	- -	- 7	1 1	- -	- 45	46 46	4m+2sm+40a 4m+2sm+40a	52 52	+ +	2 2	- -	- -	1 3
<i>Trematomus bernacchii</i> 	Adelle Land Ross Sea Weddell Sea South Shetland Islands	- - - -	7 >5 1 1	2 >11 - -	- - - -	48 48 48 48	48 48 - -	2m+2sm+44a 2m+2sm+44a 2m+2sm+44a 2m+46a	52 52 52 50	+ + - -	2 2 - -	+ - - -	+ - - -	1 3 4 6
<i>Trematomus loennbergi</i> 	Adelle Land Ross Sea, karyomorph I Ross Sea, karyomorph II	- - -	- 3 -	2 1 1	- - -	- 28 30	48 28 30	2m+2sm+44st 24m-sm+4a 22m-sm+8a	52 52 52	+ + +	2 2 2	- - -	- - -	1 3 3

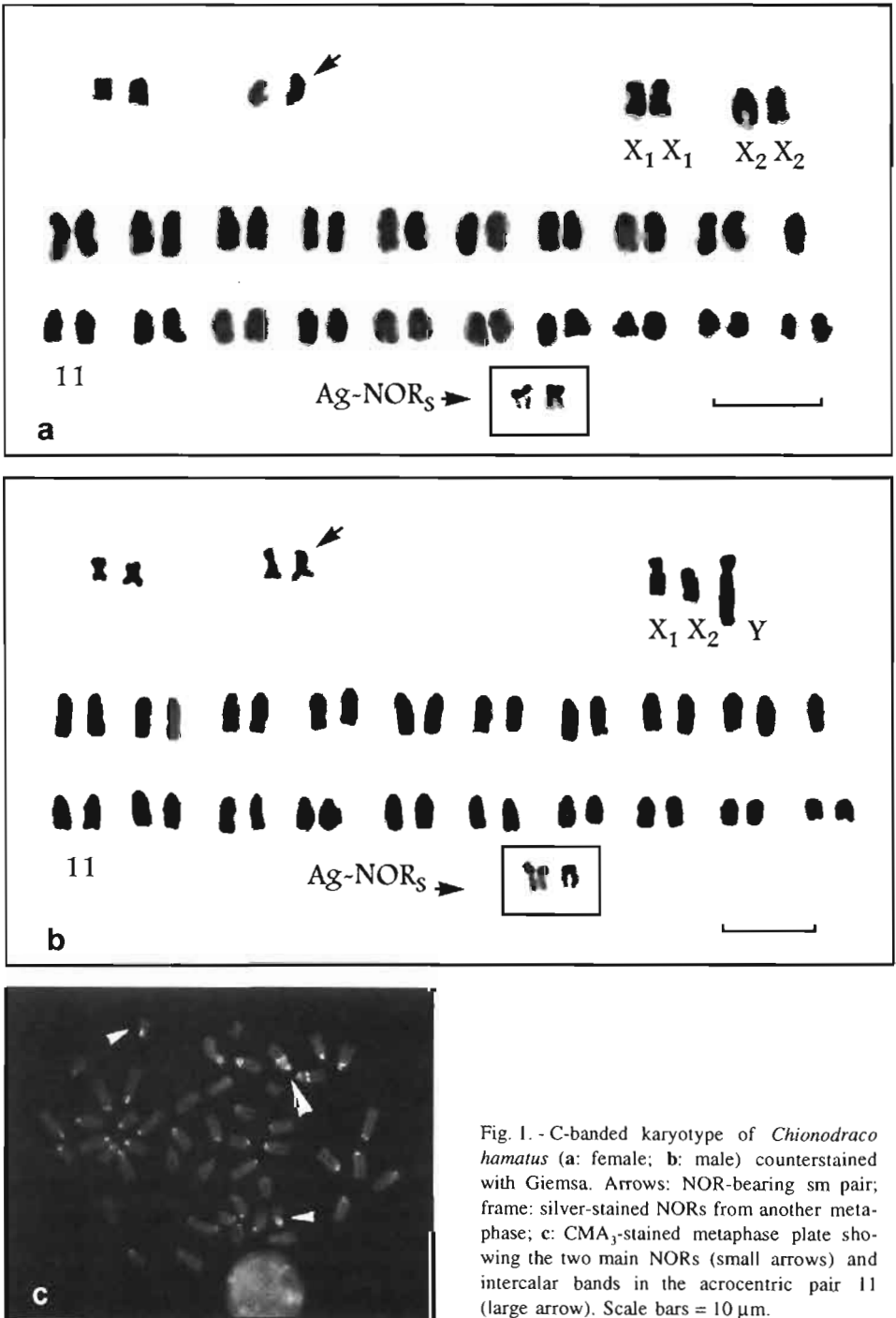


Fig. 1. - C-banded karyotype of *Chionodraco hamatus* (a: female; b: male) counterstained with Giemsa. Arrows: NOR-bearing sm pair; frame: silver-stained NORs from another metaphase; c: CMA<sub>3</sub>-stained metaphase plate showing the two main NORs (small arrows) and intercalary bands in the acrocentric pair 11 (large arrow). Scale bars = 10 µm.

$X_1X_1X_2X_2/X_1X_2Y$  (Fig. 1a,b). However, we found one specimen with a male phenotype (well developed gonads) and female-like chromosomes. Ag-staining reveals only two NORs entirely covering the short arm in a small sm pair, the NORs size being often heteromorphic within the pair. C-banding also positively stains the NORs and small heterochromatic blocks at the centromere level of nearly all chromosome pairs except the small m one, and a very clear pericentromeric interstitial band in the 11th acrocentric pair. There is also an intercalary C-band in the long arm of the Y chromosome in the male. CMA<sub>3</sub> positively stains the same blocks as C-banding, including the interstitial band which is silver-staining negative (Fig. 1c).

This species has been also karyotyped in the Weddell Sea and in the Ross Sea with similar results for chromosome number and formulae (Table I). In the Ross Sea, two males were also found showing no evidence of sex chromosome heteromorphism. Morescalchi *et al.* (1996) suggest that those fish progressively stabilize a genotypic sex-determining mechanism, which may explain this intra-specific polymorphism. C-banding and CMA<sub>3</sub> staining did not show any significant difference between the specimens of the Ross Sea and Adelie Land. Only the silver staining of NORs could be applied to material from the three localities. In the Adelie Land and Ross Sea populations, we found only two NORs while the specimens studied in the Weddell Sea had three NORs: two major NORs, situated in the same pair as those from the two other localities, and one minor NOR on the short arm of a st chromosome. Given the small sample studied, it is not possible to say if this character is widespread within the Weddell Sea population.

### **Nototheniidae**

#### ***Notothenia coriiceps***

Diploid number is  $2n = 22$  (16m + 6sm) in both males and females. Ag-NORs are located in pericentromeric position in the short arms of a large sm pair and are in some cases heteromorphic (Fig. 2a). C-banding and CMA<sub>3</sub> staining both reveal large blocks of centromeric heterochromatin (GC-rich DNA). Positive C-bands are visible on the centromeres of all chromosomes while CMA<sub>3</sub> positive bands are present in only 12 to 15 chromosome pairs (Fig. 2b).

*Notothenia coriiceps* was also karyotyped in three other Antarctic areas, providing the same chromosome numbers and slightly different formulae (Table I). Ag-NOR banding and C-banding could only be applied to the samples from Adelie Land and the Ross Sea without any obvious difference. King (1993) indicates there are constraints controlling the continuity of a metacentric karyotype (exclusively m or sm chromosomes), and argued that « once metacentricity has been attained in a lineage of species evolving by chromosome fusion, chromosomal evolution may have reached a dead end in terms of gross structural changes [...] unless the internal structure (molecular organization), it could remain metacentric in perpetuity ». In the case of *N. coriiceps*, it seems clear that fluctuations in heterochromatinization and NORs duplication without any other major change, may be responsible for the different formulae recorded in each locality. Bradley and Wichman (1994) demonstrate that radically organized genomes are associated with a high activity of rapidly evolving repetitive DNA sequences (such as the tandem repeats of centromeric constitutive heterochromatin and NORs) while conservative genomes are associated with lower abundance of such elements.

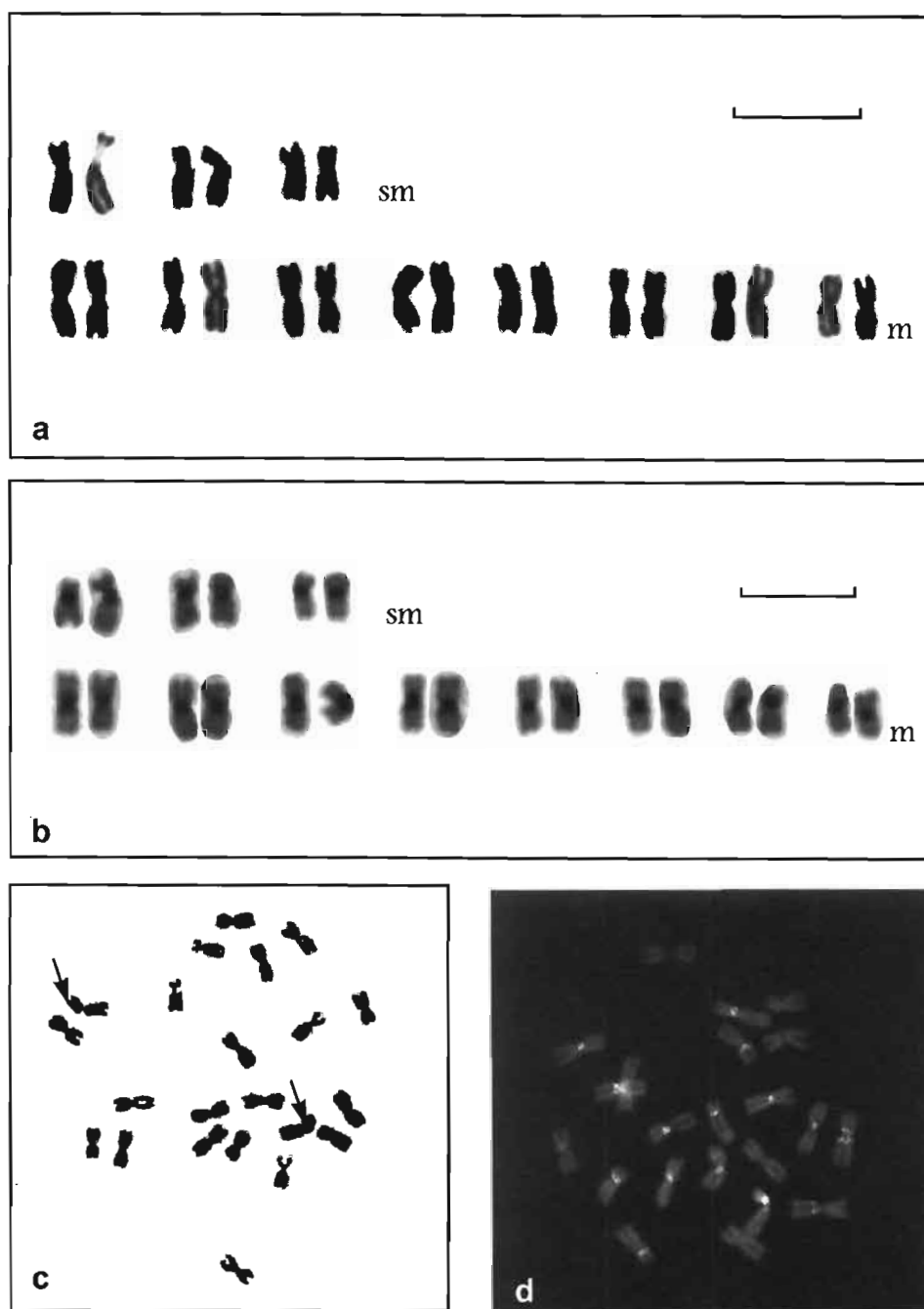


Fig. 2. - a: Giemsa-stained chromosomes of *Notothenia coriiceps*. Note the large constriction in one of the chromosomes of the first sm pair, producing an important heteromorphism; b: C-banding showing the same constriction positively stained, and large heterochromatic blocks in all centromeres; c: Ag-NOR staining in a metaphase where NORs being of equal size; d: CMA<sub>3</sub> staining showing numerous positive centromeric signals. Scale bars 10  $\mu$ m.

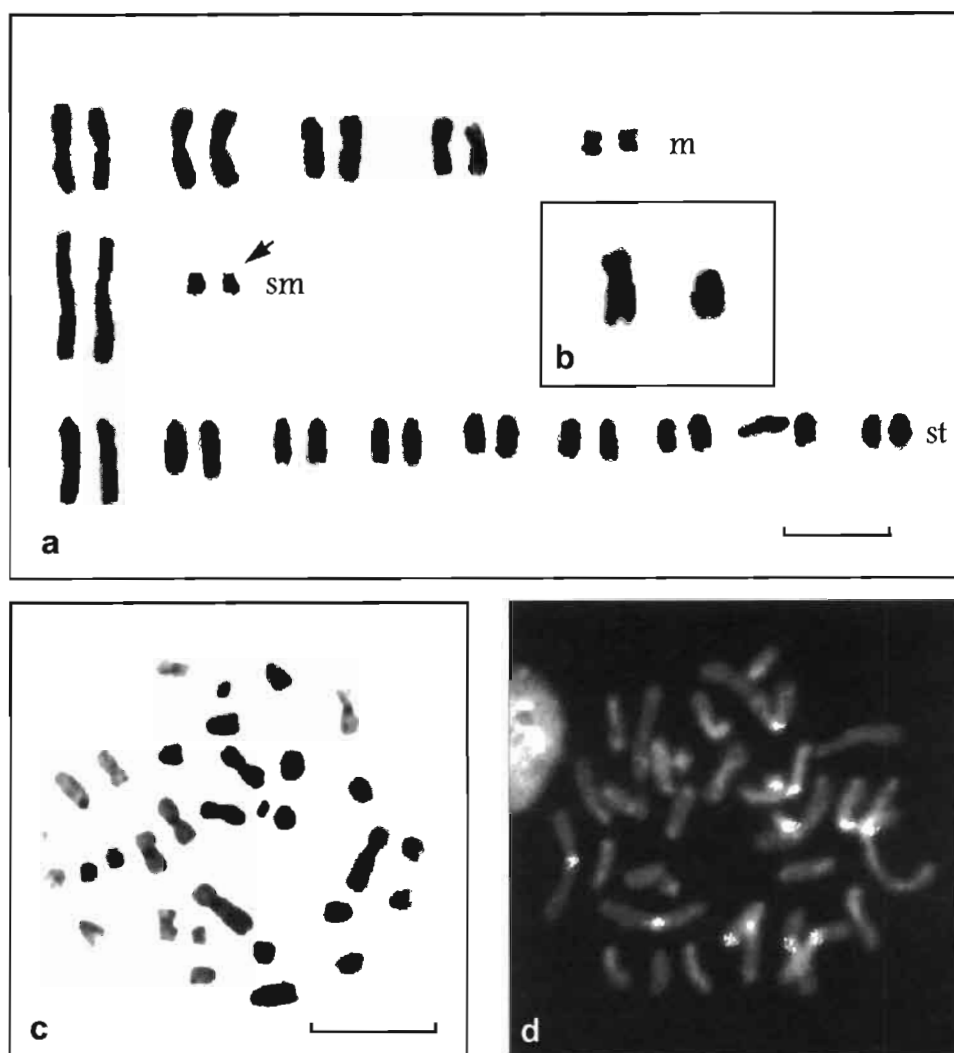


Fig. 3. - a: Ag-NOR staining counterstained with Giemsa in the chromosomes of *Trematomus pennellii* (arrows: NOR-bearing pair); b: some examples of other possible NORs locations; c: C-banding; d: CMA<sub>3</sub> staining showing positive signals on the largest chromosome pairs. Scale bars = 10  $\mu$ m.

### *Trematomus pennellii*

This result is tentative and not statistical since only two females could be examined, one giving poor quality results. Diploid number is  $2n = 32$  (14m - sm + 18a). Ag-NORs staining gave irregular results and thus we suspect intra-individual variability: only one chromosome, the smallest sm, has its entire long arm positively stained in all metaphases examined, but never the homolog. An example is given in figure 3a. But we also often found silver deposits in the pericentromeric position in one chromosome of the largest sm pair, and in intercalary position of one chromosome of the largest st pair

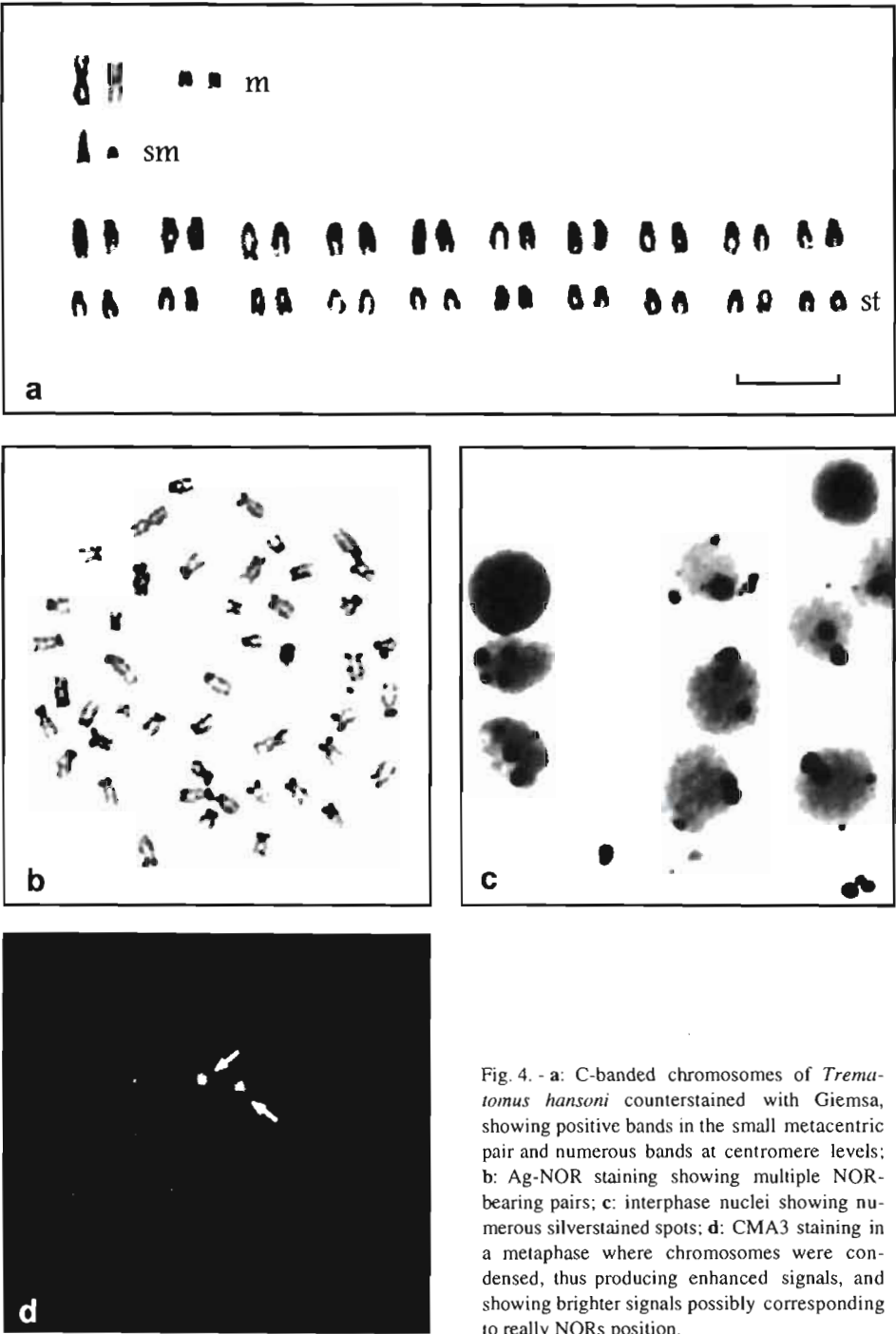


Fig. 4. - a: C-banded chromosomes of *Trematopus hansonii* counterstained with Giemsa, showing positive bands in the small metacentric pair and numerous bands at centromere levels; b: Ag-NOR staining showing multiple NOR-bearing pairs; c: interphase nuclei showing numerous silverstained spots; d: CMA3 staining in a metaphase where chromosomes were condensed, thus producing enhanced signals, and showing brighter signals possibly corresponding to really NORs position.



variability: only one chromosome, the smallest sm, has its entire long arm positively stained in all metaphases examined, but never the homolog. An example is given in figure 3a. But we also often found silver deposits in the pericentromeric position in one chromosome of the largest sm pair, and in intercalary position of one chromosome of the largest st pair (Fig. 3b). With a short barium hydroxide treatment, C-bands are small and located in centromeric position of the largest m and sm pairs (Fig. 3c). CMA<sub>3</sub> stains the same heterochromatic blocks as C-banding (Fig. 3d).

Except for NORs position (in the Ross Sea population there are only two NORs in the smallest sm pair) this species exhibited a lower intraspecific chromosomal variability than other species examined in this genus. However, regarding the small samples of specimens examined for each Antarctic region, our results have no statistical value and require additional specimens.

### *Trematomus hansonii*

Diploid number is 46 in both males and females (4m + 2sm + 40a) (Fig. 4a). The only sm pair is very heteromorphic in many metaphases. Ag-NOR staining positively marks the entire short arms of all the sm and st pairs, but there is no silver deposit in both metacentric pairs; there are frequent NORs-bearing chromosome associations, either by two or by three; interphase nuclei also show numerous silver deposits, suggesting the presence of several nucleoli (Fig. 4b,c). CMA<sub>3</sub> also produces the same pattern, although the smallest signals rapidly fade. Hence they could not be easily detected in the photographs, leaving only the two brightest ones located in the sm pair, possibly corresponding to the especially active NORs, and also small positive bands at centromere level of the metacentric chromosomes (Fig. 4d). C-banding produces small positive bands at nearly all centromere levels including those of the small metacentric pair, not the large one (Fig. 4a). This amazing number of Ag-NORs is unusual in vertebrates and, although in most cases silver stains active NORs, it is well known that various classes of heterochromatin may also be stained (Sumner, 1990). However, only *in situ* hybridization to ribosomal genes is a specific method for identifying sites of NORs; this technique is currently employed in our laboratories and the results will be published later.

*Trematomus hansonii* was studied karyologically in Adelie Land (2n = 46, no differentiated sex chromosomes, 42 Ag-NORs-bearing pairs, no chromosomal polymorphism among the seven specimens analysed); in the Ross Sea (2n = 45/46, differentiated sex chromosomes, two NORs in the two sm); in the Weddell Sea (2n = 48, female only, three NORs in the two sm and one st) and in the Bransfield Strait, Antarctic Peninsula (2n = 48, NORs location unknown) (see references and chromosome formulae in Table I). There may be some variation in establishing chromosome formulae, due to different levels of chromosome contraction, however the karyotype of *T. hansonii* is highly polymorphic, with regard to the chromosome numbers (46-48), occurrence of heteromorphic sex chromosomes and NORs numbers and location. Even if the silver-positively stained regions are not ribosomal gene clusters, they can be used as markers for population differentiation.

### *Trematomus newnesi*

Only one small female was karyotyped in Adelie Land. Diploid number is 2n = 46 (4m + 2sm + 40a). Ag-NORs are located on the short arms of the small sm pair (Fig. 5). We could not collect enough material for applying C-banding and CMA<sub>3</sub> staining since the mitotic index was very low. The specimens karyotyped in the Ross Sea have

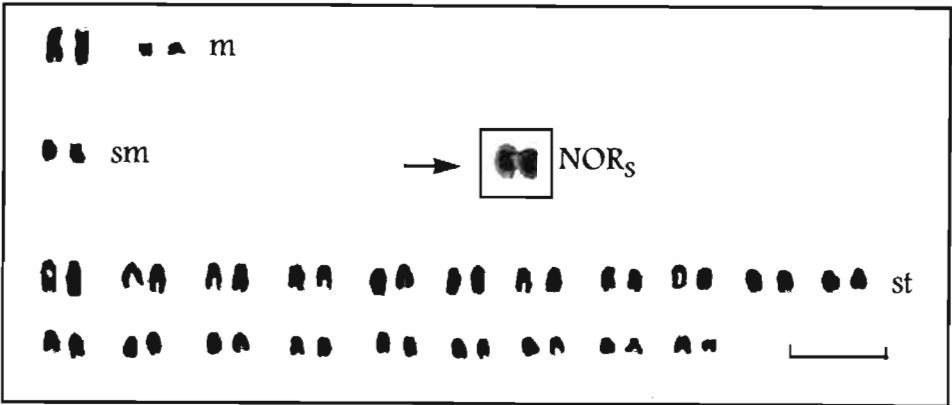


Fig. 5. - Giemsa-stained karyotype of *Trematomus newnesi*. Frame: silver stained NOR-bearing pair corresponding to the small sm one. Scale bar = 10  $\mu$ m.

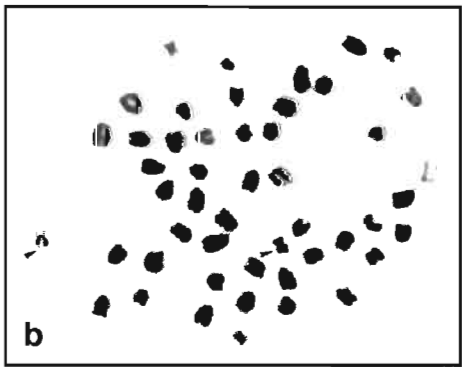
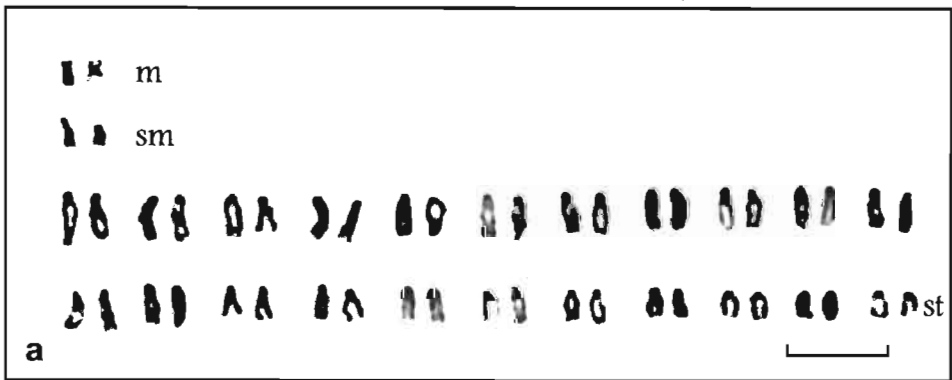


Fig. 6 - a: C-banded, Giemsa-counterstained karyotype of *Trematomus bernacchii*; b: Ag-NOR staining showing only two signals (arrows); c: CMA<sub>3</sub> staining showing NORs-bearing pair (arrows). Scale bar = 10  $\mu$ m.

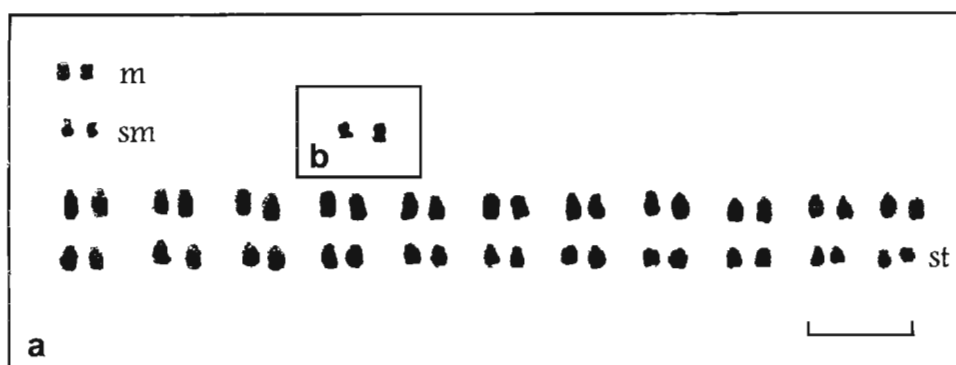


Fig. 7. - Karyotype of *Trematomus loennbergi* (a) and Ag-NORs staining of the two positively stained chromosomes (b). Scale bar = 10  $\mu$ m.

differentiated sex chromosomes which will have to be subsequently verified in Adelie Land. No significant difference between the two populations investigated could be detected.

#### *Trematomus bernacchii*

Diploid chromosome number is  $2n = 48$  ( $2m + 2sm + 44st$ ) (Fig. 6a). Ag-NORs are located in the short arms of the small *sm* pair (Fig. 6b). There are two CMA<sub>3</sub>-positive bright blocks, exactly corresponding to the Ag-NORs position, and numerous, smaller ones at centromere level of acrocentrics (Fig. 6c). C-bands are small and correspond the centromeres of all pairs except the metacentric one (Fig. 6a). There is no clear difference between the karyotypes in the Ross Sea, and Adelie Land populations and NORs are situated in the only *sm* pair which means they are probably homologous. In the Weddell Sea, the only specimen karyotyped had the same formula but Ag-NOR staining could not be applied. The single specimen studied from the South Shetland differs from the two others in chromosome formula ( $2m + 46st$ ), but the NORs location is also unknown and such data are insufficient to estimate the interpopulation variability.

#### *Trematomus loennbergi*

Only two females (one nearly dead) could be karyotyped. It is thus only possible to provide the diploid number,  $2n = 48$  ( $2m + 2sm + 44st$ ). Ag-NORs are situated in the *sm* pair (Fig. 7). In the Ross Sea, Ag-NORs are also situated in a small *sm* pair but the chromosome number is 28-30 (Table I). The three karyomorphs have the same arm number (NF = 52), so their relationships could be Robertsonian, as already suggested by Morescalchi (1992) for the two karyomorphs of the Ross Sea. This means they could be derived from each other through centric fusions ( $2n = 48, 30, 28$ ). All analysed specimens have been identified by the same specialist, but only two individuals could be studied in Adelie Land and five in the Ross Sea. Given the small sample, it is not possible to suggest an explanation for such striking variability. This stresses the necessity of a systematic revision of this species throughout its range.

## CONCLUSIONS

The Notothenioidei contain species with highly conserved karyotypes as well as species that have undergone important chromosomal changes resulting in karyotypes that are greatly reorganized. Intraspecific chromosomal polymorphism has probably been underestimated in this suborder, as is the case for many other fish groups. This is due to the small number of individuals usually analysed from one or more populations, hence failing to observe actual intraspecific variation.

This study demonstrates intraspecific variability at various levels of chromosomal organization in several species of Antarctic fishes, i.e.:

- **Changes in chromosome macrostructure (numbers and formulae)**, due to various kinds of rearrangements and/or variations of heterochromatinization. In most cases, and when we could study a sufficient sample, the rearrangements involving such changes appear to be fixed within each population studied. Unfortunately, some of the species are rare or difficult to catch, and it is hopeless to envisage a study at wider scale. For the most abundant, such as *Trematomus hansonii*, *T. bernacchii* and *Nothotenia coriiceps*, it would certainly be interesting to validate such results on larger samples and to test on the same material the genetic variability of those populations with other tools such as molecular techniques or allozyme studies. The latter were not very promising in subantarctic zones, since they displayed a low level of polymorphism, but the first studies on some Antarctic channichthyid species (Clement *et al.*, 1998) allowed identification of distinct populations and study of their differentiation.

- **Changes in chromosome microstructure (NORs localization)**. In fish, there are many reports concerning Ag-NORs (see the list in Ren & Yu, 1993). NORs size and number can vary substantially between and within species. Most publications use interspecific variation of Ag-NORs position for resolving systematic problems at various taxonomic levels. Takai and Ojima (1986), when reviewing published NOR phenotypes in fish, emphasize the NOR site diversity in some groups and consider it as an important marker for species differentiation. They also mention intraspecific variations of Ag-NORs size and number. However, Ag-NOR phenotypes are rarely used for population identification and differentiation. With Antarctic fish, we found a high rate of Ag-NORs polymorphism in two species (*T. hansonii*, *T. pennellii*), but we must first ascertain whether or not a single pattern can characterize a population.

- **Presence or absence of differentiated sex chromosomes**. Differentiated sex chromosome systems have not yet been recorded in subantarctic fish, although they have a specially high occurrence in Antarctic species. Morescalchi (1992) interpreted the latter phenomenon as an adaptation of sex determining mechanisms to the permanently cold Antarctic waters, where thermal invariability prevents a temperature-dependant sex determination. In the species where multiple sex chromosomes have been recorded, this character can be partly fixed (such as for *Chionodraco hamatus* from Adelie Land and the Ross Sea and *Trematomus hansonii* in the Ross Sea) or absent (*T. hansonii* in Adelie Land) according to the population examined. This may indicate that this process of differentiation is relatively recent. It is also interesting to note that differentiated sex chromosomes have not been recorded in those species having symmetrical karyotypes originating from multiple centric fusions. King (1993) hypothesized that chromosome fusions can have deleterious effects on fertility because there can be associations with unpaired chromosomes such as sex chromosomes, thus involving their inactivation.

Our findings call into question the general concept of the karyotype species-specificity, but not the main karyoevolutionary processes among Notothenioidei as hypothesized by Prirodina (1997) and the active process of genomic restructuring. This reinforces the idea that in Notothenioidei, chromosomal characters should be most informative for resolving systematic and phylogenetic problems at low taxonomic levels (genera, species, populations) (Ozouf-Costaz *et al.*, 1997). We can now expect similar diversification in other species, especially those with a wide geographic distribution. The geographic distribution patterns of the different karyomorphs may be useful in establishing certain biogeographical areas. In this respect, it would be interesting to examine chromosome features at the borders of such geographic areas to see whether or not a chromosomal hybrid zone can be detected. Chromosomal characters may also simply be used as markers for population identification in fish stock assessment or management.

Chromosome polymorphism patterns in fish have generally been interpreted as adaptative strategies to different hydrographic conditions (Turner *et al.*, 1985), but the differences between ecological and cytogenetical data in this adaptative context should be examined by further investigations and complementary biochemical and molecular studies. Speciation can occur in a variety of ways and it is difficult to establish whether certain chromosomal rearrangements promote speciation or whether such changes occur incidentally during or after species formation. However if chromosomal rearrangements become fixed within a population, it means that this population is partially or totally reproductively isolated from other populations in the species. If we can demonstrate in the future to what degree such changes can be fixed by karyologically studying larger samples within populations, we can expect, as suggested by Eastman (1995), to find numerous sibling species (*sensu* Knowlton, 1993) in Antarctica.

**Acknowledgements.** - This work is coordinated within the European Science Foundation Network "Fishes of the Antarctic Ocean". The Centre National de la Recherche Scientifique (CNRS, GDR 1005 and UMS 826), the Institut Français pour la Recherche et la Technologie Polaires (IFRTP) and the Italian National Program for Antarctic Research provided financial supports. We are very grateful to J. Eastman and P. Galetti for kindly reviewing this paper, to P. Lafaitte from the "Service Audiovisuel du MNHN" for preparing the figures, to J. Barthélémy for her help in table and figures presentation, and to J. Lescot for angling some of the fishes used in this study.

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## Symposium 10

### RESOURCE ASSESSMENT

Chairperson: **Guy DUHAMEL**

- BERTRAND A., JOSSE E. & J. MASSÉ. - Preliminary results of acoustic target strength measurements of bigeye (*Thunnus obesus*) and yellowfin tuna (*Thunnus albacares*). 443-450  
*Résultats préliminaires de mesures de la réponse acoustique individuelle chez le thon obèse (Thunnus obesus) et l'albacore (Thunnus albacares).*
- DUHAMEL G., MAISON J. & P. PRUVOST. - By-catch in the deep-sea longline fishery of *Dissostichus eleginoides* (Nototheniidae) from the Indian sector of the Southern Ocean. 451-458  
*Prises accessoires des pêcheries palangrières de fond de Dissostichus eleginoides (Nototheniidae) dans le secteur indien de l'Océan Austral.*
- GRANDPERRIN R., AUZENDE J.-M., HENIN C., LAFOY Y., RICHER de FORGES B., SÉRET B., VAN de BEUQUE S. & S. VIRLY. - Swath-mapping and related deep-sea trawling in the southeastern part of the economic zone of New Caledonia. 459-468  
*Cartographie et chalutages profonds dans la partie sud-orientale de la zone économique de Nouvelle-Calédonie.*





## PRELIMINARY RESULTS OF ACOUSTIC TARGET STRENGTH MEASUREMENTS OF BIGEYE (*THUNNUS OBESUS*) AND YELLOWFIN TUNA (*THUNNUS ALBACARES*)

by

Arnaud BERTRAND (1), Erwan JOSSE (1) & Jacques MASSÉ (2)

**ABSTRACT.** - Acoustics is a common method for stock assessment and to observe pelagic fish schools or individuals in their environment, but it was never applied on tuna. Knowledge about individual target strength (TS) is nevertheless the primary condition for quantitative studies and this is particularly sparse for tuna species. In this study, an original method was used to measure *in situ* acoustic target strength of 4 yellowfin tuna (*Thunnus albacares*) (4 to 30 kg) and 2 bigeye tuna (*Thunnus obesus*) (30 and 50 kg). These fish were individually caught, identified and measured, then equipped with ultrasonic tags for telemetry experiments. During the tracking operation, simultaneous underwater acoustic data were recorded with a split beam echo-sounder when the fish was inside the acoustic beam of the research vessel's echo-sounder. This experiment has shown that this method provides coherent results even if a great variability is observed. Further extensive use of this method, particularly in tuna studies, may be undertaken in the future.

**RÉSUMÉ.** - Résultats préliminaires de mesures de la réponse acoustique individuelle chez le thon obèse (*Thunnus obesus*) et l'albacore (*Thunnus albacares*).

L'acoustique est une méthode couramment utilisée pour l'estimation de stocks et l'observation directe des individus et des bancs de poissons pélagiques dans leur environnement, mais elle n'a jamais été appliquée aux thons. L'une des difficultés consiste à connaître l'indice de réflexion (TS) des individus observés et c'est particulièrement le cas pour les thons. Dans cette étude, le couplage entre le marquage acoustique et l'analyse par faisceau scindé a été utilisé pour mesurer *in situ* la réponse acoustique individuelle de 4 albacores (*Thunnus albacares*) (de 4 à 30 kg) et 2 thons obèses (*Thunnus obesus*) (de 30 et 50 kg). Ces poissons ont été pêchés individuellement, identifiés, mesurés puis équipés de marques ultrasoniques en vue d'un suivi télémétrique. Les mesures effectuées au passage du poisson dans l'axe de l'écho-sondeur du navire de recherche apportent des résultats cohérents même s'ils montrent une grande variabilité. De nombreuses applications de cette méthode peuvent être envisagées, en particulier dans l'étude des thons.

**Key-words.** - Thunnidae, *Thunnus obesus*, *Thunnus albacares*, Target strength, Acoustics, Fish tracking.

A good knowledge about individual target strength (TS) is the main prerequisite for carrying out stock assessment by acoustic methods. TS ranges of most commercially important small pelagic fish have been widely studied during the past twenty years (MacLennan and Simmond, 1992). No TS estimation has been carried out for tuna until now, except for results of Freeze and Vanselow (1986). These authors mention a range of TS data from -22 to -30 dB for large (>130 cm) bluefin tuna (*Thunnus thynnus*). However,

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these data have been estimated from theoretical considerations based on sonar observations.

Fish TS measurement is difficult, especially when the target is a fish that is vertically distributed over a large range of depths as in the case of adult tuna. A first method consists of tethered fish TS measurement (Nakken and Olsen, 1977). With such a method fish are physiologically modified and the data are not comparable with active fish responses (MacLennan and Simmond, 1992). A second method consists of TS measurement of live fish in a tank, but this method is difficult to set up for large fish. To illustrate this problem, taking into account the beam-width, a 1 m-long tuna must be at least at thirty metres below the transducer to be included in the central part of the beam. *In situ* TS measurements are considered to be the most accurate (MacLennan and Simmond, 1992). In such cases, TS measurements must be carried out on a large number of fish which have to be caught to be sampled in order to determine length and species composition. These methods are difficult to apply to scattered adult tuna because of the difficulty in catching the acoustically observed individuals.

The proposed method consists of coupling TS measurement with sonic tracking of a tagged tuna whose species and size are known. When the fish passes through the echosounder beam its acoustic response can be measured. Coupling acoustic survey with sonic tracking was used on swordfish (*Xiphias gladius*) by Carey (1990) in order to observe the fish's movement in the trophic structures, but without any attempt to measure TS.

This method was put into practice during the ECOTAP programme (study of tuna behaviour using acoustics and fishing experiments) conducted in French Polynesia by 3 research organisations: IFREMER (Institut Français de Recherche pour l'Exploitation de la MER); ORSTOM (Institut français de recherche scientifique pour le développement en coopération); and SMA (Service de la Mer et de l'Aquaculture). Tagging and tracking of yellowfin (*Thunnus albacares*) and bigeye tuna (*Thunnus obesus*) coupled with acoustic survey was undertaken. This paper presents the first results of *in situ* tuna TS measurement.

## MATERIAL AND METHODS

Experiments were carried out onboard the ORSTOM R.V. "Alis" during the ECOTAP surveys 03, 07, 15 and 18, between October 1995 and August 1997 in the Society and Tuamotu Archipelagos.

The initial purpose of these surveys was to use acoustics to study vertical and horizontal tuna movements according to their biotic and abiotic environment. During these experiments it was possible to measure the TS of the tagged tuna.

### Acoustic TS measurements

Acoustic data were collected with a SIMRAD EK500 echo-sounder connected to a 38 kHz split-beam transducer (full beam angle 6.9°) used with a pulse duration of 1.0 ms. The water column was prospected from the surface to a depth of 500 m. Acoustic and navigation data were stored via Ethernet on a PC through SIMRAD EP 500 software. The system was calibrated in accordance with the manufacturer's instructions (Simrad, 1993).

### Telemetric tracking measurements

The tracking equipment used was a VEMCO system (Shad Bay, Nova Scotia, Canada) V16P, 50 kHz, 500 and 1,000 PSI equipped with pressure sensor. Two kinds of towed

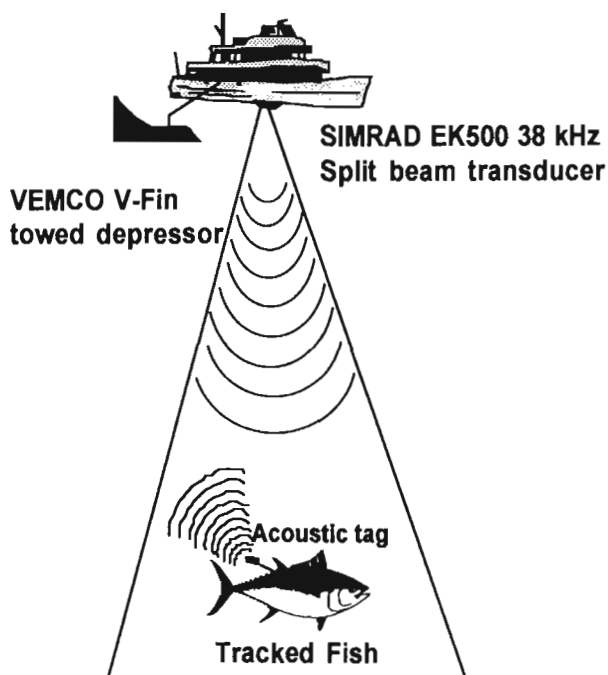


Fig. 1. - Schematic principle of target strength (TS) measurement by coupling acoustic survey and fish tracking.

hydrophones were used: a directional hydrophone VEMCO V10 and a four elements hydrophone VEMCO V41. The latter has acceptance angles in the four horizontal directions. Information was sent to a receiver system and recorded on a PC.

Tagged fish were caught by a traditional vertical handline, the drop stone fishing technique (Moarii and Leproux, 1996) or by longline onboard the R/V "Alis" and onboard professional fishing units. Depending on the fish size, two tagging methods were used. When fish were small enough, they were pulled up onboard and measured. Acoustic transmitters were fixed in the muscle of the back, close to the second dorsal fin with two nylon tie-wraps. In the case of larger fish, the acoustic tag was fixed to the anterior dorsal musculature using a tagging pole, and fish size was estimated by eye. In both cases, tuna were immediately released then tracked by the R.V. "Alis".

#### **Fish tracking and acoustic survey coupling**

The telemetric tracking system provides us the vertical and horizontal fish position in relation to the vessel. The fish was located in the echo-sounder beam when the vessel was exactly vertical to it (Fig. 1). In this case, fish TS and echo depth were automatically recorded (Fig. 2). Data validation was performed over four stages: (1) TS data were extracted with the EP 500 software at depths surrounding the tagged fish depth; (2) only echoes above -40 to -43 dB, depending on the fish size, were considered to eliminate echoes of smaller targets; (3) depth of both TS and tracked fish were compared at equal times in order to select the precise tracked fish echoes; and (4) a final control was made by looking at the paper records and EP 500 monitoring.

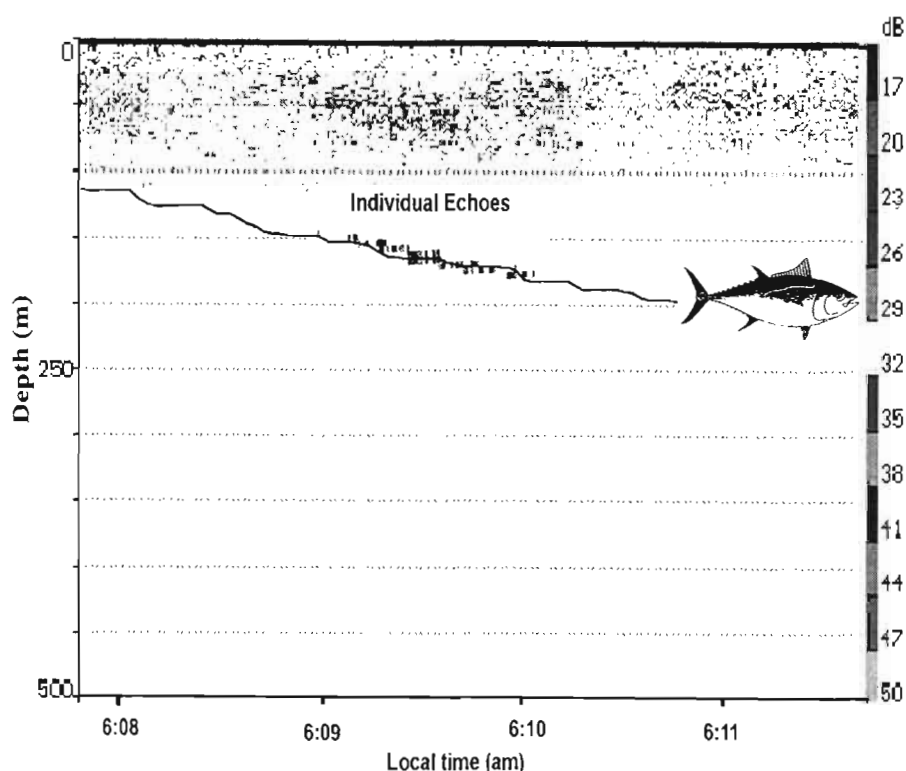


Fig. 2. - Individuals echoes (points) and depth of fish (solid line) during the track of 50 kg bigeye tuna (BE22).

#### TS of acoustic tag

Acoustics measurements verified that the tag could not produce an echo high enough to change fish TS. The values obtained were close to -50 dB. The addition of a -50 dB energy to a -30 dB energy increases the latter by only 1%. The acoustic effect of the tag has been neglected in the present study.

## RESULTS

This experiment was difficult to carry out for several reasons: (1) sonic tagging experiments necessitate a combination of optimal natural opportunities (good weather condition, availability of tuna) to be successfully undertaken (Cayré, 1991); (2) because of the beam angle (at 200 m depth, the beam diameter is about 24 m), it is unlikely that the fish will be detected acoustically; and (3) in the area of the study TS measurements could only be recorded during daytime. At night, the tracked fish moved too close to the surface to be easily detected. Furthermore, a dense scattering layer at night prevented any TS measurement with our settings.

Despite these constraints, it was possible to record 6 series of TS measurements corresponding to four yellowfin (YF10, YF13, YF14 and YF19) with an estimated weight

between 4 and 30 kg and two bigeye (BE21 and BE22) with an estimated weight between 30 and 50 kg (Table I). Individual TS varies between -34.8 and -21.4 dB (Table II). For equivalent sizes, TS is widely higher for bigeye than for yellowfin, which is in accordance with the swimbladder volume of both species (Table II). Nevertheless, TS histograms show a large variability for a same fish. Data are spread in a range of + or - 10 dB around the average (Fig. 3) with a coefficient of variation between 51.1 and 92.8% (Table II).

## DISCUSSION - CONCLUSION

This experiment, consisting of coupling fish tracking and acoustic survey, allowed *in situ* TS measurement of individually identified adult tuna. Measured data seem to be consistent as average TS increase with the fish size for a same species and with the swimbladder volume for both species. This last parameter is important because the swimbladder is supposed to be responsible for 90-95% of the backscattering energy (Foote, 1980b).

The range of TS variation exceeds 15 dB for one fish. It is well known that fish TS are widely variable (more than 20 dB) even for the same fish (Ona, 1990; MacLennan and Simmonds, 1992). Several hypotheses can explain this. Rose and Porter (1996) assumed it was due to changes in fish's swimming behaviour. Many authors such as Nakken and Olsen (1977), Foote (1980a), Foote and Ona (1985), MacLennan and Simmonds (1992), McClatchie *et al.* (1996a, 1996b) and Mukai and Ida (1996) have shown the influence of the tilt angle on TS. Nevertheless, all these kinds of experiments are usually carried out *ex situ*. *In situ* TS measurements integrate a large range of behaviour (so of tilt angle) and changes in swimbladder volume which both play an important role (Foote, 1980b; Blax-

Table I. - Characteristics of the fish tracked used in TS analysis. \*: Estimated length.

Fish No.	Survey	Species	Fishing gear	Fork length (cm)	Estimated weigh (kg)	Track duration (h)	Date of track
YF10	ECOTAP03	<i>T. albacares</i>	Drop stone	60	4	22	27-28/Oct/95
YF13	ECOTAP06	<i>T. albacares</i>	Drop stone	90	14	80	2-5/Mar/96
YF14	ECOTAP07	<i>T. albacares</i>	Drop stone	108	25	91	20-24/Avr/96
YF19	ECOTAP15	<i>T. albacares</i>	Longline	120*	30	28	16-18/Avr/97
BE21	ECOTAP15	<i>T. obesus</i>	Longline	110*	30	12	20-21/Avr/97
BE22	ECOTAP18	<i>T. obesus</i>	Longline	130*	50	33	01-03/Aug/97

Table II. - Mean TS of tracked tuna. \*: Estimated length. Data in parenthesis: Coefficient of variation in %. The swimbladder volume was estimated by the data of ECOTAP (unpubl. data).

Fish No.	Fork length (cm)	Estimated swimbladder volume (cm <sup>3</sup> )	— TS	Number of observations
YF10	60	80	-34.8 (51.1)	18
YF13	90	130	-33.0 (86.4)	102
YF14	108	215	-30.4 (92.8)	189
YF19	120*	270	-26.1 (52.2)	26
BE21	110*	1000	-24.4 (85.1)	141
BE22	130*	2500	-21.4 (60.1)	70

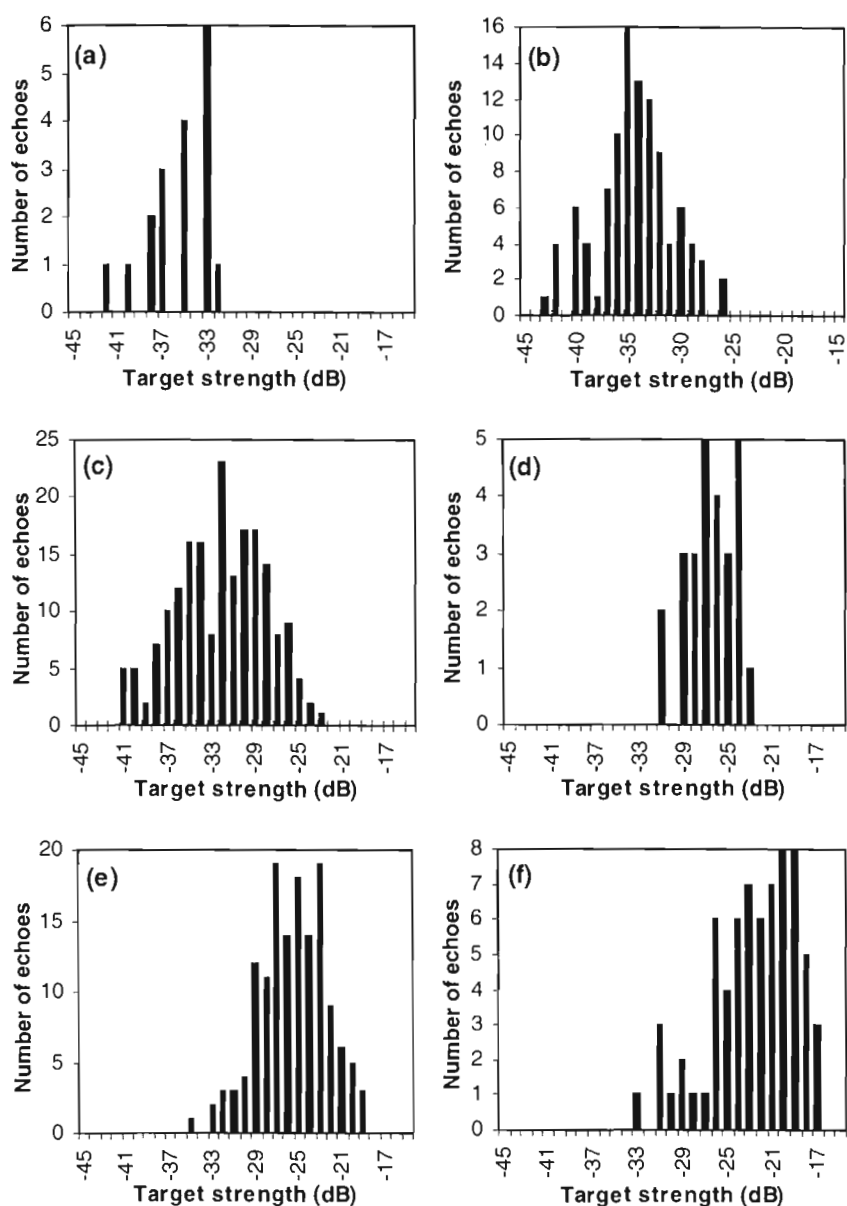


Fig. 3. - Target strength histograms of the tracked fish: Yellowfin tuna (YF) and bigeye tuna (BE). (a): YF10. (b): YF13. (c): YF14. (d): YF19. (e): BE21. (f): BE22.

ter and Batty, 1990; MacLennan and Simmonds, 1992; Misund, 1997). Classic methods of TS analysis do not allow us to determine the respective influence of these factors, which may be particularly important in the case of tuna because those fish often exhibit vertical oscillations (Holland *et al.*, 1990) changing the tilt angle.

The results presented above are insufficient to establish a reliable relationship between tuna length and TS, as it is known that it is much more difficult to establish such a relationship for organisms with a swimbladder than for fish without swimbladder (Koslow *et al.*, 1997). Nevertheless, they show that TS measurement of an identified fish swimming free in its environment is possible. Further observations with the same method, but with TS measurement as a specific objective should lead to this reliable relationship in the future. This method could be extended to other large pelagic fish as billfish.

To conclude, the specificity of this method, which allows the study of acoustic response of a fish moving freely in its environment, opens new horizons in TS research. Observation from the surface to 500 m in depth should allow us to improve our knowledge of depth influence on TS. Furthermore, the acoustic track of a tagged fish could be used to study the relationship between TS and *in situ* fish movements, while tilt angle and the effects of changes in swimbladder volume could be analysed independently. In addition this work provides the first data on *in situ* tuna TS measurements, thus could be used for future acoustic studies for tuna stock assessment.

**Acknowledgements.** - The authors wish to sincerely thank the officers and crew of the R/V "Alis" for providing valuable help during experiments. Thanks are extended to all their colleagues from IFREMER, ORSTOM and SMA, who worked within the framework of the ECOTAP programme in French Polynesia.

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## BY-CATCH IN THE DEEP-SEA LONGLINE FISHERY OF *DISSOSTICHUS ELEGINOIDES* (NOTOTHENIIDAE) FROM THE INDIAN SECTOR OF THE SOUTHERN OCEAN

by

Guy DUHAMEL, Jérôme MAISON & Patrice PRUVOST (1)

**ABSTRACT.** - The deep-sea bottom longline fishery targeting *Dissostichus eleginoides* is presently expanding everywhere in the Indian sector of the subantarctic part of the Southern Ocean. By-catch occurs in the fishery but is rarely reported because fish are often discarded. The results of two joint Japanese-French exploratory fishing cruises conducted off the Kerguelen and the Crozet islands during 1996 and 1997 showed that by-catch reaches 35% of the total number of fish and nearly 13% of the weight. The grenadier *Macrourus carinatus* (Macrouridae) is the most common species and competes with the target species in a limited depth range. Rajidae (*Raja taaf* off Crozet and *Bathyraja* spp. off Kerguelen) and Moridae (*Antimora rostrata*) are secondary by-catch. The unreported catches contribute to a loss of information for stock assessment studies which reduces the opportunity to evaluate the impact of this fishing method on the deep-sea environment. Some of these species could be successfully marketed.

**RÉSUMÉ.** - Prises accessoires des pêcheries palangrières de fond de *Dissostichus eleginoides* (Nototheniidae) dans le secteur indien de l'Océan Austral.

La pêche à la palangre de fond, dirigée sur *Dissostichus eleginoides*, est actuellement en expansion partout dans les secteurs indien et pacifique de la partie subantarctique de l'océan Austral. Des captures accessoires sont notées pour cette pêche, mais les déclarations de débarquements de ces dernières sont rares car ces poissons sont souvent rejetés à la mer. Au cours de deux campagnes exploratoires franco-japonaises de pêche profonde à la palangre, menées au large des îles Kerguelen et de Crozet en 1996 et 1997, les captures accessoires représentaient 35% du nombre total de poissons capturés et près de 13% du poids. Le grenadier *Macrourus carinatus* (Macrouridae) est l'espèce la plus commune et il concurrence l'espèce cible sur un intervalle de profondeur limité. Les Rajidae (*Raja taaf* pour Crozet et *Bathyraja* spp. pour Kerguelen) ainsi que les Moridae (*Antimora rostrata*) constituent des prises secondaires. L'absence de déclaration de ces captures entraîne une situation préjudiciable pour les études d'évaluation de stocks par perte de l'information et compromet l'évaluation de l'impact de ce mode de pêche sur l'environnement profond. Enfin, certaines de ces captures accessoires pourraient s'écouler sur les marchés avec succès.

**Key-words.** - Nototheniidae, *Dissostichus eleginoides*, Macrouridae, Rajidae, Moridae, PSE, Crozet Is., Kerguelen Is., Longline fishery, By-catch, Deep-sea.

The deep-sea bottom longline fishery for Patagonian toothfish *Dissostichus eleginoides* is expanding quickly in the subantarctic part of the Southern Ocean as a result of high value of the species on the fish market. Fishing effort is increasing mainly in the

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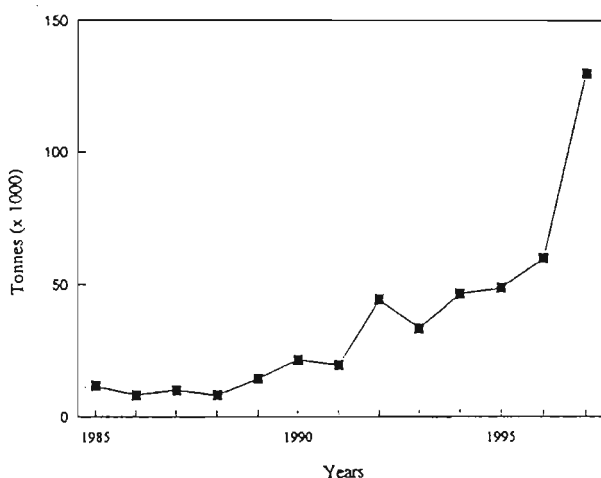


Fig. 1. - Historical world catches of *Dissostichus eleginoides* since the beginning of the directed fishery in the Southern Ocean.

Indian sector where previously only bottom trawling targeted this species. Fishing grounds occur on the slopes of the islands (Prince-Edward, Crozet, Kerguelen and Heard from the west to the east), the seamounts and in the deep-sea environment down to 1,500 m. Global landings of Patagonian toothfish were less than 20,000 tonnes annually between 1985-1989 but a steady increase is observed since then (CCAMLR, 1994; FAO, 1997) peaking during the last fishing season (1996/97) with probably more than 130,000 tonnes fished (CCAMLR, 1997) (Fig. 1), twofold of the previous one. The bulk of the recent catch comes from longliners operating in the Indian sector of the Southern Ocean. However, little information is available about the fishery because most of the vessels fish illegally inside the CCAMLR (Commission for the Conservation of the Antarctic Marine Living Resources) area. The by-catch is one of the poorly known aspects in the fishery. The opportunity to conduct exploratory deep-sea longline fishing cruises off the Kerguelen and Crozet islands during both 1996 and 1997 allowed to assess the fishing impact on the deep-sea demersal fish community.

## MATERIAL AND METHODS

Two exploratory longline fishing cruises were conducted in the Indian sector of the Southern Ocean off the Kerguelen (17 Feb.-30 Apr. 1996) and the Crozet (4 Dec. 1996-20 Apr. 1997) islands under a joint Japanese-French agreement. The longliner "ANYO MARU N°22" set bottom lines (145 and 219 respectively in each area) of 3,800/3,990 hooks and covered geographically the whole slope off the islands. The fishing method (setting time, hooks size, bait, ...) was similar during the two cruises (Duhamel *et al.*, 1997) and investigated mainly the 300-1,500 m depth range. Scientific observers, including two of the authors (J.M., P.P.) were on board and recorded all the fishing operations including the species catch. The number of specimens of each species was reported for each line and, in the case of the Crozet cruise, the weight of fish was also available.

Species were identified using the most recent revision of Antarctic ichthyofauna (Gon and Heemstra, 1990). Rare species were collected and deepfrozen for further identification. The specimens of *Bathyraja* from the Kerguelen area were not identified to species level because most of the specimens were discarded before hauling on board, so only some sets were followed to the species level (*B. eatonii*, *B. irrassa* and rare *B. murrayi*). Concerning the Macrouridae family, the need to revise the *Macrourus* genus was noted because specimens were difficult to allocate to the poorly understood complex of species *carinatus*, *holotrachys*, *whitsoni*. Consequently all the specimens have been assigned to the species described in the area (Iwamoto, 1990): *M. carinatus*. Furthermore the analysis did not include the invertebrate by-catch which occurred frequently off the Crozet islands (chiefly the subantarctic *Lithodes murrayi* and the red stone *Paralomis aculeata* crabs) but only rarely off the Kerguelen islands (only *Paralomis aculeata* on a seamount off the shelf). These observations agreed with the geographic distribution described by Amaud (1987).

An index of abundance using the catch per unit effort (in number of specimens for 100 hooks per fishing hour) was established to compare the results for each species and analyse their depth occurrence. Mean abundance for each 100 meter depth interval was used to illustrate the tendencies. Values were considered in the analysis only for the depth intervals grouping more than 5 sets.

Finally random sampling of specimens was used to draw up the length frequency distributions of the most abundant species off the Crozet islands. Some data were previously available for the Kerguelen area (Duhamel *et al.*, 1997). The total length (TL) to the centimeter below was recorded.

## RESULTS

The catches of the two exploratory deep-sea longline cruises show that the target species, *D. eleginoides*, reaches a very similar percentage (65% in number) of the total catches in these two well separated geographical areas of this part of the Indian sector of the Southern Ocean (Table I). The percent by mass of this species is up to 87% of the total weight (Crozet). By-catch includes nine families of demersal fish (14 species) and one of the bathypelagic group (Stomiidae, one species). Some differences are observed in the species composition between the two areas but by-catch is dominated in number by three families: the Macrouridae (genus *Macrourus*), the Rajidae (genera *Bathyraja* and *Raja*) and the Moridae (genus *Antimora*). The first family seems to be a very important by-catch for both areas (23 and 27% in number for Kerguelen and Crozet respectively) in the investigated depth range.

The depth occurrence of the main species shows that *Macrourus carinatus* appears at about 550 m and its abundance peaks before 1,000 m and decrease afterwards (Fig. 2a). Trends at Crozet and Kerguelen are similar but with a slight shift. *Antimora rostrata*, is nearly absent at depths shallower than 700 m and exhibits a constant increase in abundance to about 1400 m (Fig. 2b), but mean values do not exceed 0.12 specimens/100 hooks/fishing hour. Lower values are always noted off the Kerguelen islands. Finally, in the case of the skates, *Raja taaf* off the Crozet islands shows irregular trends and low values at various depths. The species is already noted from the lower limit of the depth range. Abundance increases to 800 m and observed maximum values are between 800 and about 1,100 m then from 1,300 to 1,500 m (Fig. 2c). High values in abundance

of *Bathyraja* spp. off the Kerguelen islands are observed between 500-600 m, medium values between 600-1,000 m and 1,100-1,300 m (Fig. 2c). The Moridae and Rajidae are always less abundant (maximum 0.25/0.35 specimens/100 hooks/fishing hour) than the targeted *D. eleginoides* or the by-catch *M. carinatus* (maximum values: 2.5 and 1.5 respectively).

Samples of all the abundant by-catch species off Crozet islands have an incomplete length frequency distribution. Almost no specimens under 30 cm of *A. rostrata* (Fig. 3a) or 40 cm of *M. carinatus* (Fig. 3b) and *R. taaf* (Fig. 3c) are noted. Size range reaches about 65 cm for the first species, 80 cm for the others. Multimodal distributions are recorded for some of these (*A. rostrata* and *R. taaf*).

Table I. - Fish species composition in exploratory deep-sea longline catches for *Dissostichus eleginoides* off the Kerguelen and the Crozet islands during 1996 and 1997.

Area Period Fishing depth (m) Number of longline sets Number of hooks	Crozet Islands 4 Dec. 1996 - 20 Apr. 1997 317 - 1466 219 865260				Kerguelen Islands 17 Feb. 1996 - 30 Apr. 1996 300 - 1700 145 544553	
	No.	%	Weight (kgs)	%	No.	%
<b>Lamnidae</b>						
<i>Lamna nasus</i>					3	0.005
<b>Squalidae</b>						
<i>Etmopterus granulosus</i>					38	0.06
<i>Somniosus microcephalus</i>					1	0.002
<b>Rajidae</b>						
<i>Bathyraja eatonii</i> + <i>irrasa</i>					6760	11.22
<i>Raja taaf</i>	3393	3.57	7862	2.05		
<b>Synphobranchidae</b>						
<i>Histiobranchus bathybius</i>	2	0/002				
<b>Stomiidae</b>						
<i>Stomias gracilis</i>	2	0.002				
<b>Muraenolepidae</b>						
<i>Muraenolepis marmoratus</i>	16	0.017			3	0.005
<b>Moridae</b>						
<i>Antimora rostrata</i>	3158	3.32	3460	0.90	746	1.24
<i>Lepidion</i> sp.	7	0.007				
<b>Macrouridae</b>						
<i>Macrourus carinatus</i>	26077	27.43	37946	9.84	14028	23.28
<b>Nototheniidae</b>						
<i>Dissostichus eleginoides</i>	62400	65.64	333353	87.12	38663	64.15
<i>Lepidonotothen squamifrons</i>	6	0.006			24	0.04
<i>L. larseni</i>	3	0.003				
<b>Bothidae</b>						
<i>Mancopsetta maculata</i>	1	0.001				
<b>Total</b>	95065	100	382621	100	60266	100

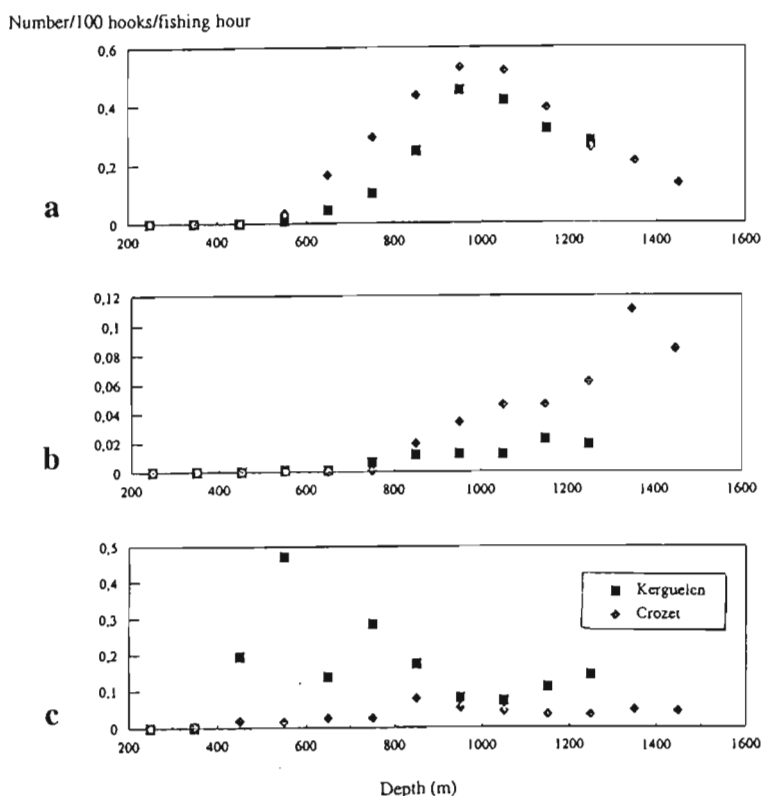


Fig. 2. - Relative abundance of by-catch (number of fish for 100 hooks per fishing hour) in relation to the depth (in meters) for all longline sets carried out during the exploratory deep-sea fishing cruises off the Kerguelen (1996) and the Crozet (1996/97) islands. a: *Macrourus carinatus*. b: *Antimora rostrata*. c: *Bathyraja* spp. (Kerguelen) and *Raja taaf* (Crozet).

## DISCUSSION

The occurrence of by-catch species in the present deep-sea exploratory longline fishing agrees with the known geographical distribution of the species (Duhamel, 1987; Gon and Heemstra, 1990) in each surveyed area. The only known shark (*Somniosus microcephalus*) off the Crozet islands has been recorded recently (Duhamel, 1997) and its absence in the longline catches is not surprising. Indeed, comparatively, even at Kerguelen (Table I) where the species is common, only one catch was noted. The difference in species of Rajidae from Crozet (genus *Raja*) and Kerguelen (genus *Bathyraja*) is also well established and no overlap is presently known. The eastern limit of distribution of *Lepidonotothen larseni* in the Crozet islands excludes the species from Kerguelen islands. The results show that the by-catch species with a wide range of geographical distribution such as *A. rostrata* and *M. carinatus* have a similar proportion by numbers in the catches in both areas (1.2/3.3% and 23.3/27.4% respectively) as well as for the target species *D. eleginoides* (64.2/65.6%). This suggests adaptation to a similar ecosystem in this range of the deep-sea environment. The main observed difference concerns the relative propor-

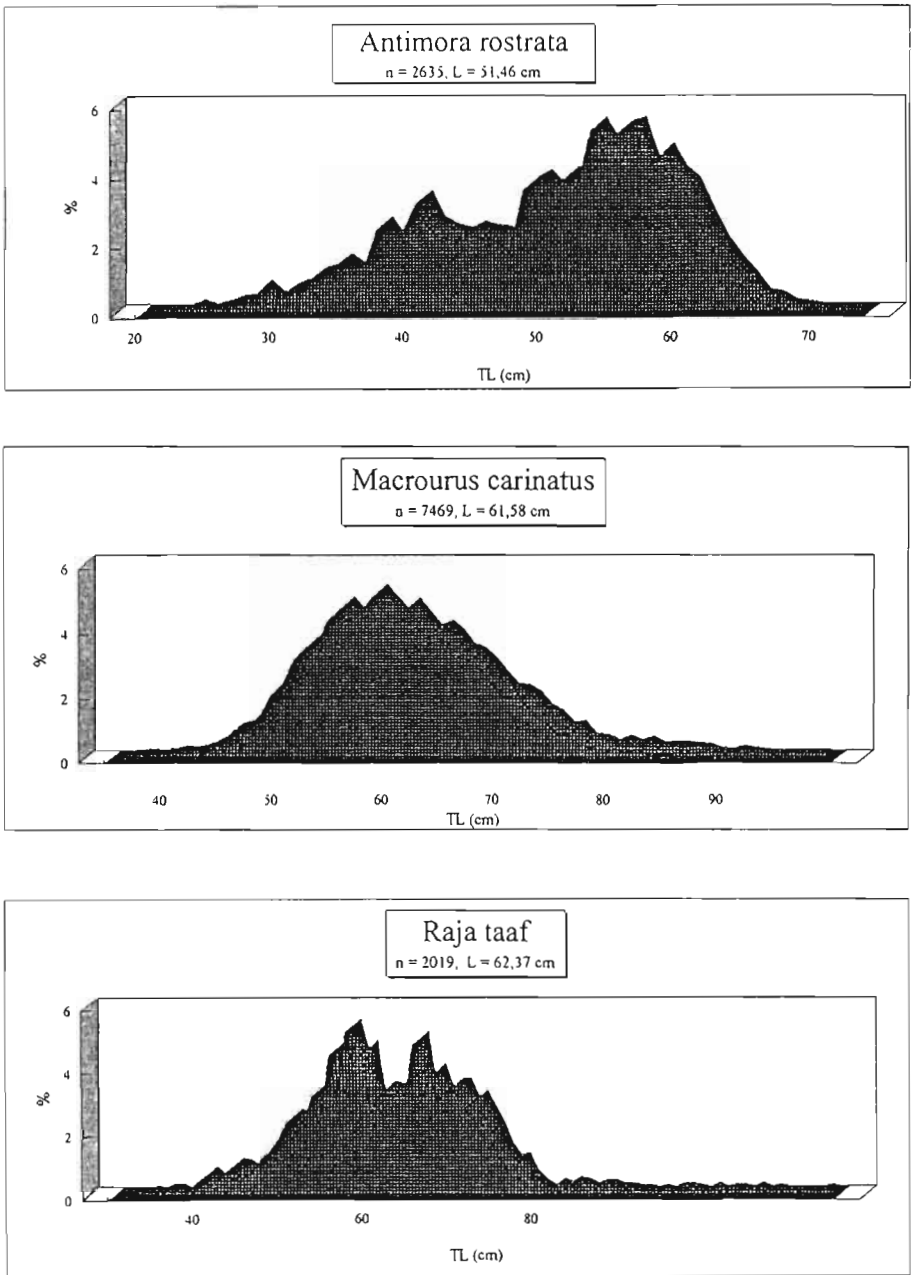


Fig. 3. - Length frequency distribution of *Antimora rostrata*., *Macrourus carinatus* and *Raja taaf* caught during the exploratory deep-sea fishing off the Crozet islands (1996/97).

tion of the Rajidae between the two areas. The genus *Bathyraja* off Kerguelen seems to be more abundant (11.2%) that the genus *Raja* (3.6%) off Crozet, but it is necessary to con-

sider that the first genus includes three species of which two occur in the deep sea (*B. eatonii*, *B. irrasa*) against the second one which is monospecific. The large area of the Kerguelen slope compared to the Crozet slope (up to tenfold) could explain the difference in the diversity of this part of the deep-sea ichthyofauna from such a bathymetric range.

Few species can be considered as important by-catch from these results of exploratory deep-sea longline fishing cruises. They belong to families Macrouridae, Rajidae and Moridae and are the same as those reported in other areas where a longline fishery of *D. eleginoides* occurs, such as South Georgia (SC-CAMLR, 1995) in the Atlantic sector of the Southern Ocean. The percentage of by-catch observed in this study, both in number and weight, suggests that if these levels were applied to the commercial long line fishery for *D. eleginoides* (both legal and illegal), a total by-catch of between 5 and 10,000 tons would have been taken in the 1996/97 season. Statistical reports (SC-CAMLR, 1997) include few landings of by-catch species, but the available observers' reports (SC-CAMLR, 1995) corroborate the results of the present study. This underlines the potential impact of the long line fishery on the deep-sea ichthyofauna and indicates that there is a high level of discarded catch that is unreported.

Similar trends in abundance off the Kerguelen and the Crozet islands are noticeable when the occurrences by depth of *M. carinatus* and *A. rostrata* (Fig. 2a, b) are analyzed. The first species exhibits a bathymetric distribution between 550 and probably 1550 m with a maximum occurrence close to 1,000 m. The second species lives in deeper water and the depth range of sampling does not allow the limits of the depth distribution to be determined. Grey (1956) indicates that this species is abundant in the 800-1,800 m range. Conversely Rajidae seem to be the most abundant by-catch in the shallow longline sets. The observed differences for the Rajidae between the two zones could be explained by contributions of the various species (one species off Crozet, two species off Kerguelen). However the *Bathyraja* genus from Kerguelen is more abundant than *Raja* from Crozet in the shallow range, even if the two *Bathyraja* species contribute to the values. The bimodal abundance off Kerguelen is probably related to the species distribution with, firstly, *B. eatonii* and secondly *B. irrasa* (Duhamel *et al.*, 1997).

Comparing the relative abundance of the by-catch species with that of the target species, only *M. carinatus* reaches a level of a by-catch to be able to compete with *D. eleginoides* in the two areas. The values are even close to those of *D. eleginoides* in the middle range of the present depth occurrence study. The catch per unit effort standardized with a generalised additive model (Hastie and Tibshirani, 1990) shows clearly that the two species compete for hooks (SC-CAMLR, 1997). Grenadiers may have the strongest effect on *D. eleginoides* yield at depths between 800 and 1,000 m. Consequently such depths need to be avoided if the fishery wants to target mainly *D. eleginoides*.

Finally the length frequency distributions demonstrate that there is probably a hook selectivity excluding the small specimens in each species. Size range is similar between the Crozet (present data) and Kerguelen (Duhamel *et al.*, 1997) areas for the common species *M. carinatus* and *A. rostrata*. *R. taaf* seems to be a small species when length frequency distribution is compared to those of *B. eatonii* and *B. irrasa*. The total amount of the by-catch indicates it should be of commercial interest, especially in the case of grenadiers and skates, for which there is a market. The fish are of sufficient size, and of good taste, to be converted to other fish products and it is regrettable that these fish are often discarded. Because the by-catch fish are dead when discarded, there is a considerable impact of this type of bottom long lining on the deep-sea species, and such utilisation would in part compensate for this.

**Acknowledgements.** - The captain and crew of "Anyo Maru N° 22" are thanked for their cooperative assistance during the two cruises. Special thanks are given to the two other observers, P. Bletterie and S. Takada, for their help on board. We are grateful to two reviewers of the manuscript for their useful comments and to their contribution to improve the English language.

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## SWATH-MAPPING AND RELATED DEEP-SEA TRAWLING IN THE SOUTHEASTERN PART OF THE ECONOMIC ZONE OF NEW CALEDONIA

by

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**ABSTRACT.** - Within the framework of the programme "ZoNéCo" of evaluation of the marine resources of the economic zone of New Caledonia, a series of operations were completed in the south-eastern part of the economic zone. The first was a bathymetrical and geophysical survey of the major part of the Norfolk Ridge and the southern end of the Loyalty Ridge. The data obtained on this survey provided a base for the preparation and completion of the deep-sea trawling survey "HALIPRO 2", the main objective of which was to search for commercial quantities of deep-sea fish, primarily orange roughy (*Hoplostethus atlanticus*). During this survey, 106 hauls were made between 230 and 1,860 m depth. A total catch of 263 fish species was made belonging to 192 genera and 101 families. In particular, 37 species of sharks and rays were collected of which 40% are new to science. The results confirm the extreme specific richness of the deep-sea ichthyofauna and the presence of species of commercial interest such as the alfoncino, *Beryx splendens*. However, orange roughy, was not located.

**RÉSUMÉ.** - Cartographie et chalutages profonds dans la partie sud-orientale de la zone économique de Nouvelle-Calédonie.

Dans le cadre du programme "ZoNéCo" d'évaluation des ressources marines de la zone économique de Nouvelle-Calédonie, une série d'opérations a été réalisée dans la partie sud-est de cette zone. Dans un premier temps, un relevé bathymétrique et géophysique a couvert la presque totalité de la Ride de Norfolk et de l'extrémité méridionale de la Ride des Loyautés. Les données acquises ont ensuite servi de base à la programmation et à la réalisation de la campagne de chalutage exploratoire "HALIPRO 2" menée sur des fonds de 230 à 1 860 m de profondeur et dont le principal objectif était d'identifier des ressources exploitables en poissons de profondeur, et notamment l'empereur (*Hoplostethus atlanticus*). Durant cette campagne, 106 traits de chalut ont permis la capture de 263 espèces de poissons appartenant à 192 genres et 101 familles. En particulier 37 espèces de requins et de raies ont été collectées dont 40% étaient inconnues. Les résultats de cette campagne confirment la grande richesse spécifique de l'ichtyofaune profonde et la présence d'espèces d'intérêt commercial, notamment de *Beryx splendens*. En revanche, aucun empereur n'a été capturé.

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Key-words. - Deep-sea fishes, *Beryx splendens*, ISEW, New Caledonia, Swath-mapping, Deep-sea trawling, Inventory.

In order to evaluate the living and non-living marine resources of the economic zone (EZ) of New Caledonia, a programme named "ZoNéCo" was initiated in 1991. "ZoNéCo" comprises several partners: the French State, the "Service Hydrographique et Océanographique de la Marine" (SHOM), "Météo-France", the Territory of New Caledonia, the three Provinces ("Iles Loyauté", "Nord" and "Sud"), and the locally established research institutions (IFREMER, ORSTOM, "Université Française du Pacifique"). This programme involves many scientific disciplines: bathymetry and imagery of the seafloor, gravimetry, magnetism, seismology, satellite altimetry, physical oceanography and fishery sciences. The first priority of "ZoNéCo" was to establish a precise bathymetric map of the New Caledonian EZ. Initially all available conventional bathymetric data were compiled and a map of the entire EZ (Missègue *et al.*, 1992) was produced (Fig. 1). This map was used to select areas to be covered in more detail by swath-mapping surveys. Between 1993 and 1996, four swath-mapping cruises were carried out onboard the French research vessel "L'Atalante" equipped with the multibeam echosounder EM 12 DUAL. One of the major results of these surveys was the discovery of a number of ridges and seamounts previously unknown as well as the acquisition of more precise bathymetry of named structures. These data were used to select the features that could potentially be favorable to the existence of deep sea living resources. As a result of this work, several exploratory fishing surveys were carried out onboard the ORSTOM research vessel "Alis". In 1994 and 1995 two of these surveys were devoted to deep bottom-longline fishing between 300 and 800 m north of the main island of New Caledonia and on the Loyalty Ridge (Grandperrin *et al.*, 1995a, 1995b). In 1994, a bottom-trawling survey investigated the deep resources along the east coast and in the south of New Caledonia between 200 and 1,200 m (Grandperrin *et al.*, 1995c).

As far as the southeastern part of the EZ is concerned, several seamounts were studied by scientific exploratory cruises between 1980 and 1987 (Barro, 1981; Richer de Forges and Grandperrin, 1987) and then exploited by longlining from 1988 to 1991 (Laboute, 1989; Grandperrin *et al.*, 1990; Grandperrin and Lehodey, 1993; Lehodey, 1994) targeting the deep demersal alfoncino, *Beryx splendens*. The objective of this paper is to illustrate the input of swath-mapping on the strategy of the first deep-sea trawling survey ("HALIPRO 2") carried out in 1996 in the southeastern part of the EZ in the frame of the "ZoNéCo" programme. As a result of this work, commercial fishing is expected to take place in this area in the forthcoming years.

### Topography of the southeastern part of New Caledonia

The southeastern part of New Caledonia EZ was swath-mapped in 1993 during the "ZoNéCo 1" survey onboard "L'Atalante" (Pautot *et al.*, 1993; Dupont *et al.*, 1995). Fifty-six EM 12 DUAL bathymetric profiles allowed a quite complete coverage of the area. A central zone represented by a 2,400 m-deep basin separating the Norfolk Ridge to the west from the Loyalty Ridge to the east was excluded of the survey.

The Norfolk Ridge, underlined by the isobath 2,000 m, is trending N 160°E and is characterized by a succession of ridges and seamounts. In detail the structure of the Norfolk Ridge comprised a west and east region. The western region is characterized by 2,000 m-deep ridges supporting shallow seamounts culminating at less than 600 m-

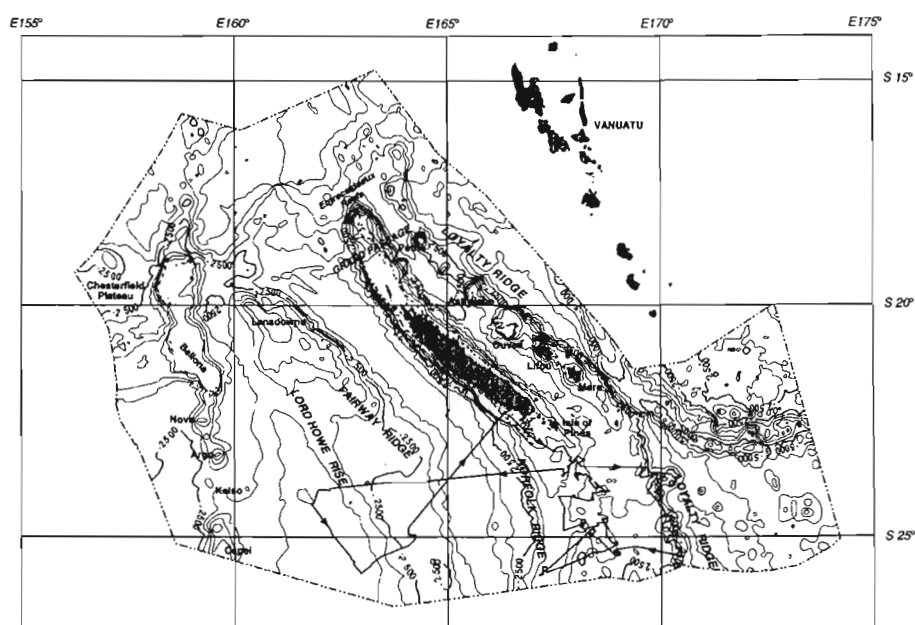


Fig. 1. - Bathymetric map of the EZ of New Caledonia (after Missègue *et al.*, 1992) and tracks of the deep-sea trawling survey "HALIPRO 2" (from Grandperrin *et al.*, 1997b).

depth. These seamounts are elongated along a NS or N 160°E direction and show small narrow tops of about 5 km long bounded by steep slopes (30%). The eastern region is deeper and comprises three large mounds 30 to 40 km-wide, aligned in a N 160°E trend.

Although the central basin east of the Norfolk Ridge was not surveyed during the cruise "ZoNéCo 1", the conventional bathymetric data give a general idea of its topography. It is 2,500 m-deep in its southern part and then the depth rises toward the northwest up to 23°S where a less than 2,000 m-deep saddle separates the central basin from the Loyalty Basin.

East of the central basin, the Loyalty Ridge is globally deeper than the Norfolk Ridge and its base is underlined by the 2,200 m isobath. The Loyalty Ridge is like the Norfolk Ridge roughly trending N 160°E, but the seamounts and ridges present on the main ridge show a complicated pattern, probably related to recent volcanic and tectonic events. Schematically they are aligned along a zig-zag model with the succession of NS and N 150°E directions. The secondary ridges are about 60 km-long and 40 km-wide and are flanked by steep slopes. They have large tabular summits culminating at less than 1,000 m depth. On the eastern side of the area transverse N 160°E trending ridges are evident. They are related to a transverse faulting characterizing the whole area.

The morphology and the nature of the structures observed on the swath-mapping and imagery maps offer particularly interesting features as far as marine habitats are concerned. Before swath-mapping, the surface exploited by the fishery from 1988 to 1991 on both the Norfolk and the Loyalty Ridges at depths ranging from 500 to 800 m was about 280 km<sup>2</sup>. The survey "ZoNéCo 1" increased this surface up to 1,230 km<sup>2</sup>. The depths between 800 to 1,500 m occupy about 15,000 km<sup>2</sup>.

## A SURVEY FOR DEEP-SEA RESOURCES: HALIPRO 2

The deep-sea trawling survey "HALIPRO 2" (Grandperrin *et al.*, 1997a, 1997b) was carried out in 1996 onboard the "*Tangaroa*", a New Zealand research vessel owned by the National Institute of Water and Atmospheric Research Ltd. (NIWA) and chartered by the programme "ZoNéCo". The survey aim was the identification of deep-sea fishery resources mainly on the Norfolk Ridge and the southern end of the Loyalty Ridge. This zone, the area of which is 73,000 km<sup>2</sup>, was previously mapped during the seabed mapping survey "ZoNéCo 1" (Pautot *et al.*, 1993). An area to the west of the Lord Howe Rise was also surveyed (Fig. 1).

### The survey

The trawl was similar to the one used by the New Zealand commercial deep-sea trawlers (headline length 38 m, headline height 7 m, codend meshsize 100 mm) with an additional codend liner/cover meshsize 40 mm. Seventeen scientists were on board, representing the three Provinces of New Caledonia, IFREMER, ORSTOM, and research institutions from New Zealand, Great Britain and the United States. In addition to fishing operations, the marine environment was studied by obtaining 18 CTD temperature and salinity profiles down to 1,500 m.

A total of 3,755 nautical miles were covered and 106 hauls were made at depths ranging from 230 to 1,860 m of the survey area. Most of the tows were completed on flanks and tops of seamounts (Fig. 2). The charts produced during the swath-mapping survey "ZoNéCo 1" were entered into the navigation system of the "*Tangaroa*" and they proved remarkably useful, providing such details that they were used by the fishing officers and scientists to work out the best trawl track. During a large part of the survey they could have been used as navigation charts.

### The non-living environment

During "HALIPRO 2", temperature and salinity profiles did not fluctuate between stations. The corresponding TS diagrams show that the Intermediate Antarctic Waters as defined by Reid (1965, 1973), i.e., with temperature between 2°C and 10°C and salinity between 33.8 and 34.5, do not reach the EZ of New Caledonia (Grandperrin *et al.*, 1997b). Temperature and salinity sections also show a geostrophic circulation from east to west in the north of the zone and from west to east in the south.

Bottom types varied according to the area. Sediment was collected on many occasions and large pieces of rock were often found in the trawl. Seamounts from the northern part of the southern end of the Loyalty Ridge have a hard substratum with little sediment. Further south, sandy sediment appears more frequent. Although the seamounts of the Norfolk Ridge show mostly hard substratum outcrops, they are easier to trawl than the ones of the Loyalty Ridge for their summits and flanks are predominantly covered with mud and sand. As a whole, the area surveyed by "HALIPRO 2" was rather difficult to investigate by bottom-trawling due to the multiplicity of hard and rough bottoms on seamounts, particularly on sharp peaks and pinnacles.

### The catch

"HALIPRO 2" confirmed the high fish species diversity already suspected from previous results (Barro, 1981; Richer de Forges and Grandperrin, 1987; Grandperrin *et al.*, 1990; Grandperrin and Lehodey, 1993; Lehodey, 1994; Séret *et al.*, 1997). A total of 263 fish species belonging to 192 genera and 101 families were caught (Table I). Of signifi-

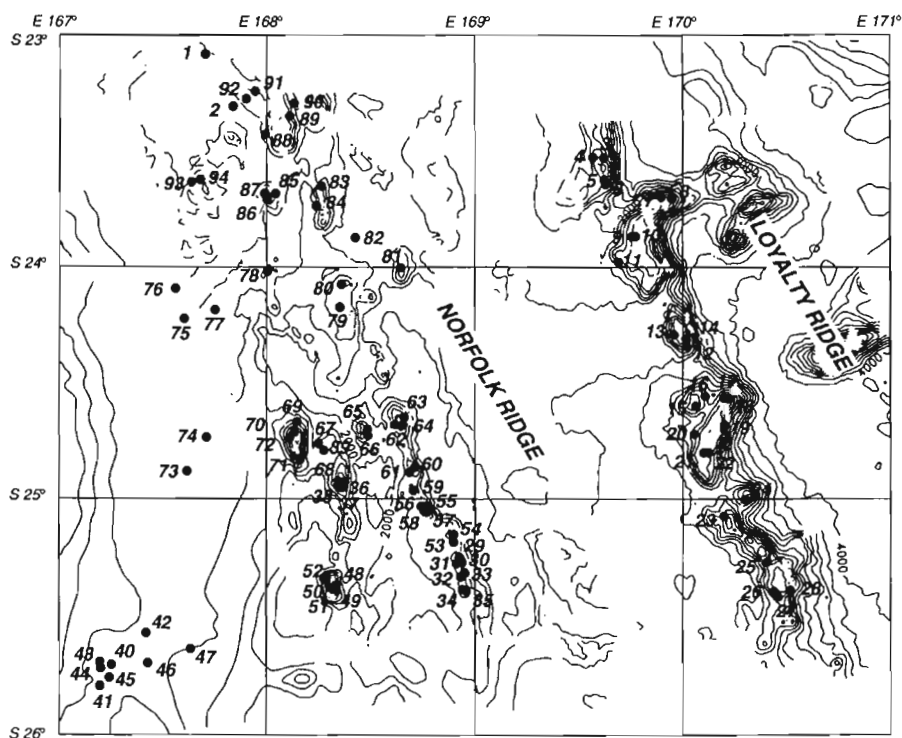


Fig. 2. - Swath-bathymetric map of the south-eastern part of the EEZ of New Caledonia and locations of deep-sea trawling stations completed on the Norfolk Ridge and the southern part of the Loyalty Ridge during the deep-sea trawling survey "HALIPRO 2". For practical reasons, the trawled seamounts had to be named during the survey so that their name could be entered into the R/V "Tangaroa" internal computer network. These names have not been submitted to the agreement of the Intergovernmental Oceanographic Commission. Stations numbers are given in Grandperrin *et al.* (1997b). Interval between isobaths: 200 m.

cance, 37 different species of shark and ray were collected of which 40% are new to science (Table II). The best represented families were the Macrouridae with 24 species, the Squalidae with 19 species and the Alepocephalidae with 16 species. These families are found between 500 and 1,500 m in the world ocean. Many samples were collected for further analysis (population genetics, phylogeny, ageing, tissue analysis and parasite identifications).

A total of 15 commercial species representing close to 88% of the total fish catch were caught: *Beryx splendens*, *B. decadactylus*, *Pseudopentaceros richardsoni*, *Pentaceros decacanthus*, *Pristipomoides auricilla*, *P. flavipinnis*, *Cookeolus japonicus*, *Etelis coruscans*, *Wattsia mossambica*, *Hyperoglyphe antarctica*, *Seriola dumerili*, *S. lalandi* and the sharks *Squalus megalops*, *S. mitsukurii* and *Squalus* sp. *Beryx splendens* comprised 94% of the commercial fish. Its presence was confirmed between 500 and 800 m over the summits of the seamounts where this species was previously exploited. Hauls made deeper over slopes and flat bottoms produced no catch of orange roughy in spite of the fact that this species is abundant further south where it is the target of a flourishing New Zealand fishing industry.

Many invertebrates were caught such as echinoderms (ophiuroids and crinoids), molluscs (a few squids and octopus) and crustaceans. Many benthic organisms (sponges, gorgonians and corals) were often found meshed in the net. Among the crustaceans, some species of prawns which are commercially exploited in other countries were found in the catches (*Aristeomorpha foliacea*, *Aristeus virilis*, *Heterocarpus* spp., ...).

Table 1. - Families (101) and numbers of genera (192) and species (263) of fishes caught during the deep-sea trawling survey "HALIPRO 2" off New Caledonia.

	Nb genera	Nb species		Nb genera	Nb species		Nb genera	Nb species
<b>Chondrichthyes</b>			Chlorophthalmidae	4	7	Zeniontidae	1	1
Chlamydoselachidae	1	1	Scopelarchidae	1	1	Macrurocyttidae	1	1
Hexanchidae	1	1	Notosudidae	2	2	Grammicolepididae	2	2
Squalidae	8	16	Synodontidae	1	2	Fistulariidae	1	1
Odontaspidae	1	1	Giganturidae	1	1	Macrohamphosidae	1	1
Scyliorhinidae	2	8	Paralepididae	3	3	Scorpaenidae	2	3
Proscylliidae	1	1	Anopteridae	1	1	Triglidae	1	1
Rajidae	2	3	Evermannellidae	1	1	Psychrolutidae	1	1
Urolophidae	2	2	Omosudidae	1	1	Liparidae	1	1
Hexatrygonidae	1	1	Alepisauridae	1	3	Caproidae	1	2
Chimaeridae	2	2	Myctophidae	4	5	Priacanthidae	1	1
Rhinochimaeridae	1	1	Neoscopelidae	1	2	Serranidae	1	1
<b>Teleostei</b>			Moridae	3	3	Apogonidae	1	1
Congridae	1	1	Melanonidae	1	1	Acropomatidae	2	3
Ophichthyidae	1	1	Macrouridae	13	24	Lutjanidae	2	4
Synphobranchidae	2	2	Ophidiidae	4	7	Lethrinidae	1	1
Nettastomidae	1	1	Lophiidae	1	1	Pentacerotidae	3	4
Serrivomeridae	1	1	Chaunacidae	2	3	Bramidae	3	3
Nemichthyidae	2	3	Ogcocephalidae	2	2	Emmelichthyidae	2	2
Halosauridae	3	5	Himantolophidae	1	1	Carangidae	2	3
Gonorynchidae	1	1	Linophrynidae	2	2	Bathyclupeidae	1	1
Argentiniidae	1	1	Melanocetidae	1	1	Chiasmodontidae	3	5
Eurypharyngidae	1	1	Gigantactinidae	1	1	Uranoscopidae	1	1
Bathylagidae	1	1	Veliferidae	1	1	Percophidae	1	2
Alepocephalidae	9	16	Trachipteridae	1	1	Scombrobrachidae	1	1
Stomiidae	3	4	Berycidae	3	5	Gempylidae	8	9
Chauliodontidae	1	1	Ostracoberycidae	1	1	Trichiuridae	2	2
Astronesthidae	2	3	Trachichthyidae	1	2	Centrolophidae	2	2
Idiacanthidae	1	2	Anomalopidae	1	1	Arionmmidae	1	1
Malacosteidae	2	2	Diretmidae	1	1	Monacanthidae	1	2
Melanostomidae	5	6	Holocentridae	1	1	Triodontidae	1	1
Phonichthyidae	2	2	Stephanoberycidae	1	1	Tetraodontidae	1	1
Gonostomidae	2	4	Polymixiidae	1	1	Triacanthotidae	4	4
Sternopyrichidae	3	4	Barbouriidae	1	1			
Aulopidae	1	1	Zeidae	3	3	Total	192	263

Table II. - Chondrichthyan fishes caught during the deep-sea trawling survey "HALIPRO 2" off New Caledonia (L &amp; S refers to Last and Stevens, 1994).

<b>Chlamydoselachidae</b>
<i>Chlamydoselachus anguineus</i> Garman, 1884
<b>Hexanchidae</b>
<i>Hepiranchias perlo</i> (Bonnaterre, 1788)
<b>Squalidae</b>
<i>Centrophorus harrissoni</i> McCulloch, 1915 (Séret & Last, in prep.)
<i>Centrophorus</i> sp. n. "white fins" (Séret & Last, in prep.)
<i>Centroscymnus owstoni</i> Garman, 1906
<i>Centroscymnus "plunketi"</i> (Waite, 1909)
<i>Dalatias licha</i> Rafinesque, 1810
<i>Deania quadrispinosa</i> (McCulloch, 1915)
<i>Etmopterus lucifer</i> Jordan & Snyder, 1902
<i>Etmopterus</i> sp. cf. sp. "C" of L & S (1994) (Séret & Last, in prep.)
<i>Etmopterus</i> sp. cf. sp. "D" of L & S (1994) (Séret & Last, in prep.)
<i>Etmopterus</i> sp. cf. sp. "F" of L & S (1994) (Séret & Last, in prep.)
<i>Etmopterus</i> sp. ("pseudo-squaliolus") (Séret & Last, in prep.)
<i>Isistius brasiliensis</i> (Quoy & Gaimard, 1824)
<i>Squaliolus laticaudus</i> Smith & Radcliffe, 1912
<i>Squalus megalops</i> (Macleay, 1881)
<i>Squalus mitsukurii</i> Jordan & Snyder, 1903
<i>Squalus</i> sp. n. (Séret & Last, in prep.)
<b>Odontaspidae</b>
<i>Odontaspis ferox</i> (Risso, 1810)
<b>Scyliorhinidae</b>
<i>Apristurus</i> sp. 1 (long-snout) (Nakaya & Séret, in prep.)
<i>Apristurus</i> sp. 2 (white type) (Nakaya & Séret, in prep.)
<i>Apristurus macrorhynchus</i> (Tanaka, 1909)
<i>Apristurus platyrhynchus</i> (Tanaka, 1909)
<i>Apristurus sinensis</i> Chu & Hu, 1981
<i>Apristurus</i> sp. cf. <i>microps</i> Gilchrist, 1922 (Nakaya & Séret, in prep.)
<i>Parmaturus</i> sp. 1 (Séret & Last, in prep.)
<i>Parmaturus</i> sp. 2 ? (Séret & Last, in prep.)
<b>Proscyllidae</b>
<i>Gollum attenuatus</i> (Garrick, 1954)
<b>Rajidae</b>
<i>Raja</i> (subgenus C) sp. n. (blue) (Séret & Last, in prep.)
<i>Pavoraja</i> sp. 1 (dark) (Séret & Last, in prep.)
<i>Pavoraja</i> sp. 2 (pale) (Séret & Last, in prep.)
<b>Urolophidae</b>
<i>Plesiobatis daviesi</i> (Wallace, 1967)
<i>Urolophus</i> sp. n. (Séret & Last, in prep.)
<b>Hexatrygonidae</b>
<i>Hexatrygon</i> sp. n. (long snout) (Séret & Last, in prep.)
<b>Chimaeridae</b>
<i>Chimaera phantasma</i> Jordan & Snyder, 1900
<i>Hydrolagus</i> sp. (Whitley, 1939) (Dagit & Séret, in prep.)
<b>Rhinochimaeridae</b>
<i>Rhinochimaera pacifica</i> (Mitsukuri, 1895)

## DISCUSSION - CONCLUSION

The emergence of multibeam echosounders has greatly improved seafloor mapping. These tools allow a more efficient coverage of large areas of ocean than the conventional sounders. They also provide images of the bottom which could be interpreted in terms of seabed texture. These kinds of data, which help to describe the topography and bottom type, provide a necessary step prior to any exploratory surveys and subsequent commercial fishing trips. Knowledge of these parameters saves time during the survey and increases the safety of deep-sea trawling operations. The topography of the southeastern part of the EZ of New Caledonia is now well known. The area is characterized by large shallow ridges which should constitute good spots for deep-sea longlining and trawling operations. However, swath-mapping has revealed the existence of sharp peaks and pinacles which make trawling difficult.

The survey "HALIPRO 2" confirmed the high richness of the fish fauna and the presence of an exploitable resource mainly represented by the alfoncino. Unfortunately, the expected orange roughy was not caught. The absence of this fish could be related to the stratigraphy of the water column where the Intermediate Antarctic Waters do not occur. In contrast, the Intermediate Antarctic Waters are present around New Zealand where they characterise the habitat of the orange roughy.

During the "ZoNéCo 1" survey of R/V "L'Atalante" in June and July 1993, an Acoustic Doppler Current profiler (ADCP) revealed a large counter-clockwise gyre centered between the two ridges of Norfolk and the Loyalty Islands and recorded as deep as 700 m (Henin, 1994). The existence of the geostrophic currents flowing in opposite directions revealed during "HALIPRO 2" might confirm the existence of the gyre. Such a strong eddy might play a major role in the dissemination of larvae and juveniles of some marine species of commercial interest such as *Beryx splendens* (Lehodey, 1994; Lehodey *et al.*, 1997) and would evidence the existence of a single stock over the area. This information is of major importance for the management of a future fishery. Genetic studies presently carried on muscle samples of alfoncino collected during "HALIPRO 2" will confirm or not this hypothesis.

**Acknowledgements.** - The authors wish to thank the programme "ZoNéCo" of evaluation of the marine resources of the economic zone of New Caledonia for its financial and logistical support. Special thanks are due to Dominique Dagit, Tomio Iwamoto, Peter Last, Nigel Merrett, Kazuhiro Nakaya and Clive Roberts for their fruitful collaboration in identifying and studying some groups of fishes.

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## Symposium 11

### FISH FARMING, AQUARIUM FISH ISSUES

Chairperson: **Roland BILLARD**

- BILLARD R. - Biological diversity in pond fish culture. 471-479  
*Diversité biologique en pisciculture d'étang.*
- SAMANTARAY K., MOHANTY S.S., DASH G. & K. MISHRA. - The effects of 481-490  
acclimation temperature on the growth performance and the activities of  
aspartate and alanine aminotransferase of the air-breathing teleost,  
*Channa striata* (Channidae).  
*Les effets de la température d'acclimation sur la croissance et l'activité  
de l'aspartate et de l'alanine aminotransférases chez le téléostéen à respi-  
ration aérienne, Channa striata (Channidae).*



## BIOLOGICAL DIVERSITY IN POND FISH CULTURE

by

Roland BILLARD (1)

**ABSTRACT.** - Aquaculture has taken advantage of the large number of fish species and their diversity. Over a total of 25,000 species more than 150 are cultivated at least for a part of their cycle but less than ten, mostly cyprinids and salmonids, yield 90% of the world production. There are now many attempts to rear new species either to compensate for over-exploitation of stocks (cod, tuna, ornamental fish, ...) or for conservation purpose (endangered species). Finally the number of cultivated fish reflects the diversity of the market demand traditionally linked to the diversity of fish caught by fisheries. Man is also taking advantage of the fish physiological diversity via association of species: for instance the introduction of police fish to eliminate unwanted small fish from ponds or the so-called polyculture practised in China. In that case in intensively manured ponds, in inland waters, several species, especially cyprinids, exhibiting complementary feeding habits, are stocked and consume food at different levels of the trophic web. The most commonly used species are the silver carp *Hypophthalmichthys molitrix* feeding on microalgae and microzooplankton, the grass carp *Ctenopharyngodon idella* on macrophytes, the bighead *Aristichthys nobilis* and the common carp *Cyprinus carpio* on zoobenthos and macrozooplankton. Other species are also introduced, taking advantage of other niches such as the black carp *Mylopharyngodon piceus* which eats molluscs. Similar systems are operating in India with the Indian major carps and in all cases the diversity of bacteria and plankton contribute to the productivity. The habitat is sometimes diversified, for example acadja or rice field. These systems are deeply integrated with agriculture and rural life and represent an efficient way of recycling wastes (integrated fish farming); they also contribute to the development of original landscape and to the stability of human societies. The productivity is high, in average more than 3 tons of fish/ha/year in China. In intensively manured polyculture systems the main productivity is due to a limited number of associations of bacteria, plankton and fish but they were selected out from a much wider range of species and ecological complex.

**RÉSUMÉ.** - Diversité biologique en pisciculture d'étang.

L'aquaculture fait appel à un grand nombre d'espèces de poissons et à leur diversité biologique. Sur un total de 25 000 espèces plus de 150 font l'objet d'élevage au moins durant une partie de leur cycle vital, mais moins d'une dizaine d'espèces, surtout cyprinidés et salmonidés, assurent 90% de la production aquacole mondiale. Des essais d'élevage de nouvelles espèces sont pratiqués pour compenser la surexploitation par pêche (morue, thons, poissons d'ornement) et pour la conservation d'espèces. Il est en réalité fait appel dans de nombreux cas à la diversité physiologique des poissons. Par exemple l'utilisation, dans des étangs, de poissons piscivores (police fish) pour éliminer de jeunes poissons introduits accidentellement ou résultant d'une reproduction non contrôlée. Un exemple de l'utilisation de la diversité physiologique des poissons est aussi illustré par la polyculture telle qu'elle est pratiquée en Chine où sont associées, dans un même étang, plusieurs espèces inféodées à différents niveaux du réseau trophique: la carpe argentée s'alimentant de phytoplancton, la carpe herbivore de macrophytes, la carpe à grosse tête et la carpe commune de zoobenthos et de macrozooplankton. Des associations d'espèces sont aussi pratiquées en Inde avec les "major Indian carps" et la "culture composite". Ces systèmes reçoivent une forte fertilisation organique et présentent des productivités élevées (>3 t/ha/an par exemple en Chine). Dans tous les cas, la production bénéficie de la diversité microbienne et plan-

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tonique. Il y a d'autre part des diversifications d'espèces combinées à des diversifications d'habitat et d'environnement (acadjas, rizipisciculture). Ces systèmes sont fortement intégrés à l'agriculture et contribuent au cadre de vie et à la stabilité des sociétés humaines locales. Il faut souligner que de telles associations d'espèces, en particulier celles qui mettent en oeuvre des cyprinidés, se traduisent par une production mondiale annuelle de 9 millions de tonnes, c'est-à-dire plus de 60% de la production aquacole totale. Dans ces systèmes intégrés, la productivité est finalement due à un nombre très limité d'espèces, que ce soit de poissons ou de leurs proies, mais ces espèces ont été sélectionnées directement ou indirectement à partir du pool plus large d'espèces et de complexes écologiques qui constitue la biodiversité.

Key-words. - Cyprinids, Biodiversity, Aquaculture, Ponds, Polyculture.

"Biodiversity" may be simply defined as the totality of the genetic material of living organisms and the ecological complex in which they live. A more complete definition was given by Wilson (1992) as: « ... the variety of organisms considered at all levels, from genetic variants belonging to the same species through arrays of species to arrays of genera, families, and still higher taxonomic levels, includes the variety of ecosystems, which comprise both the communities of organisms within particular habitats and the physical conditions under which they live ». According to Beveridge *et al.* (1994) it is implicit, in Wilson's definition, that the concept of biodiversity be extended to include abiotic components of the environment upon which plants and animals depend and that it is the most appropriate interpretation with regard to aquaculture. Pullin (1996) considers as very important the microbial and planktonic biodiversity in many aquaculture operations.

The present minireview will be restricted to the uses of the biological diversity in pond fish culture which offer good examples of some traditional association of fish yielding presently more than 60% of the total production of the world aquaculture.

### THE NUMBER OF CULTIVATED SPECIES

The associations of species were made possible by the large number of fish species (more than 25,000) and their biological diversity, especially their feeding habits. More than 150 fish species are presently cultivated, at least during a part of their live cycle (FAO, 1994). In a single country, like India, Tripathi (1990) listed 70 species (Table I). There are now many attempts to rear new species (cod, tuna, ...) to compensate for over-exploitation of stocks or for conservation purpose (endangered species such as sturgeons), and in the case of ornamental fish captured in the wild. Finally the large number of cultivated fish reflects the diversity of fish caught by fisheries and offered to the market; the consumer is expecting some diversity of species and products from aquaculture. The biodiversity in terms of food production should then include the commodity chain, the consumer and the societies.

It should be pointed out that only a limited number of species are fully domesticated, i.e., when all phase of the life span are controlled and when fish are genetically adapted to new environments imposed by man (Table II). They belong mainly to the groups of primitive euteleosts (ostariophysi, salmoniformes) and some perciformes. A few species have been reared for long periods of time, for example the common carp, *Cyprinus carpio*, cultured from thousands years in China and hundred years in Europe. The

reproduction of carp was fully controlled in hatchery only recently (Woynarovich, 1962); before the broodfish were placed in ponds where natural spawning occurred and the fry were collected several weeks or months later. These simple technologies allowed however some massal selection (Wohlfarth and Hulata, 1989) and the production of strains adapted to specific climatic conditions or to disease resistance (Kirpichnikov *et al.*, 1993).

The rainbow trout *Oncorhynchus mykiss* is another well-domesticated species. Its culture started in the middle of the 19th century after the procedure for artificial reproduction has been established (it was in fact for brown trout *Salmo trutta*) in France (Billard, 1989). Common carp and rainbow trout are probably the most intensively domesticated of

Table I. - Number of fish per family suitable for culture in warm freshwater ponds and tanks in India (after Tripathi, 1990). Six species are exotic.

Megalopidae	1	Siluridae	3	Mugilidae	2
Clupeidae	2	Bagridae	5	Ophiocephalidae	3
Chanidae	1	Schilbeidae	3	Cichlidae	2
Notopteridae	2	Sacchobanchidae	1	Anabantidae	2
Cyprinidae	41	Anguillidae	1	Mastacembelidae	1
Total					71

Table II. - State of domestication of cultured fish (after Wohlfarth and Hulata, 1989).

<b>Acipenseriformes</b>	Wild stocks taken in hatcheries for artificial reproduction and stocking juveniles.
<b>Cypriniformes</b>	The common carp is the most intensively domesticated species; several strains are identified for example « big belly » in China, Galician and Ukrainian strains in Europe, the DOR 70 hybrid in Israel, the ornamental koi in Japan.  Chinese carps are now currently propagated in hatcheries in China for a small number of generations but there was no major selective breeding programme so far.  Major indian carps, catla, rohu, mrigal. Induced spawning has been carried out in hatcheries for a limited number of generations.
<b>Siluriformes</b>	<u>Channel catfish</u> . Large number of domesticated strains with relatively small number of generations. Several closely related species are also cultivated, blue and white catfish, some bullheads.  <u>Southern Asia catfish</u> . Artificial reproduction has been recently reported for <i>Pangasius</i> .
<b>Chanidae</b>	Milkfish is still wild and its culture depends on captured juveniles
<b>Salmoniformes</b>	The reproduction and first rearing of salmonids are relatively easy due to spontaneous ovulation and spermiation (no need for hormonal treatments) and to the large size of eggs (4 to 10 mm). Many strains were established by sampling natural populations from different river systems and reproduction in hatcheries. Rainbow trout <i>Oncorhynchus mykiss</i> is highly domesticated with a large number of generation for many hatchery strains. Atlantic salmon <i>Salmo salar</i> is recently domesticated with few generations for several populations. Several salmonids or salmoniformes species are propagated in hatcheries for stocking: Pacific salmon, brown trout, Arctic char, lake trout, cutthroat trout, ayu,...
<b>Perciformes</b>	Cichlids: several species of « tilapias » are now extensively produced in Africa (where they originate) and in Asia. Some selective breeding programme have started with tilapia. Groupers ( <i>Epinephelus</i> ) Carangidae ( <i>Seriola</i> ) and Mugilidae (mulets) start to be cultivated but are still wild as they still depend on captured juveniles.

all fish species. They were the first in which gene transfer was carried out (review in Depêche and Billard, 1994; Sin, 1997). The goldfish *Carassius auratus* has been reproduced and grown in captivity in China for 1,000 years as ornamental fish.

Domestication of the other cultivated fish is more recent. The process of domestication was relatively fast, about 15-20 years for Atlantic salmon *Salmo salar*, sea bass *Dicentrarchus labrax*, sea bream *Sparus aurata* and *Pagrus major* (Foscarini, 1988), channel catfish *Ictalurus punctatus* and tilapias, especially *Oreochromis niloticus*. The process of domestication was strongly accelerated by research programmes and was realised by the combined efforts of fish farmers and scientists. The first step of rearing often starts in laboratories, for example *Epinephelus aeneus* (Hassin *et al.*, 1997). In most of these species domestication resulted from the reproduction in hatcheries of specific stocks isolated from wild populations with still a low number of generations. Genetic changes were observed as well as changes in behaviour (Ruzzante, 1994; Tsukamoto *et al.*, 1997). Domestication may also result rapidly in loss of genetic variability (Agnèse *et al.*, 1995). A large variety of fish species and populations exhibiting various degree of genetic variability are introduced in the diverse production systems; this component of the biodiversity is quite well controlled by man.

Several species, yellowtail *Seriola quinqueradiata* and milkfish *Chanos chanos* which world production exceeds respectively 150,000 and 350,000 tons/year and some mullets are still wild and their culture consists simply in catching juveniles in the sea and growing them in cages (yellowtail) or in tambacs (milkfish). These systems are rather common in SE Asia and increasing difficulties are reported in catching juveniles. In these systems the control by man is much lower and the genetic individual variability of the populations of wild fry have been submitted to an entirely different pressure of selection than domesticated hatchery fry. In the case of the milkfish, Hilomen-Garcia (1997) has shown that hatchery bred milkfish fry and juveniles show major morphological abnormalities by comparison to wild fry.

### Association of fish species in pond culture

#### *Association for a better use of the trophic web (concept of polyculture)*

The culture of a single species does not usually allow to take advantage of the diversity of food available in ponds: plankton, benthos, macrophytes, organic wastes (with however the exception of omnivorous fish such as some tilapias). This is why some production systems associate several fish species having complementary feeding habits. The functioning of the system and its efficiency on a short term basis is then based partly on the number of fish species but mostly on their physiology and morphology. One of the most famous association is the Chinese carp system. This association includes the silver carp *Hypophthalmichthys molitrix* feeding on microalgae and macrozooplankton, the grass carp *Ctenopharyngodon idella* on macrophytes, the bighead *Aristichthys nobilis* and the common carp on zoobenthos and macrozooplankton. Other species are also introduced taking advantage of other niches such as the black carp *Mylopharyngodon piceus* which eats molluscs. Similar systems including the so-called composite culture are operating in India with the major Indian carps (Tripathi, 1990; Nandeesa, 1995). In that case 3 species are associated: *Catla catla* feeding on plankton (64% crustaceans, 30% micro-algae), *Labeo rohita* taking periphyton, rotten vegetation and *Cirrhinus mrigala* consuming various detritus. The "Chinese carps" are also added with 3 other species *Pangasius pan-*



*gasius*, *Ompok bimaculatus*, *Notopterus chilata*, feeding respectively on molluscs, insects and weedfish (Jhingran, 1986). These systems are deeply integrated with agriculture and they represent an efficient way of recycling wastes (integrated fish farming). The productivity is high (in average more than 3 tons of fish/ha/year in China) which is much higher than in any other system of meat production consuming only primary production (grass).

Sometimes association of species is combined with diversification of habitat by the use of bamboo (acadja) (Hem and Avit, 1994). Another example is given by rice-fish farming systems which are quite common all over Asia (de la Cruz *et al.*, 1992; Haroon and Pittman, 1997) and expanding (Little *et al.*, 1996). Ghosh (1992) reviewed several systems and reported associations of more than 30 species. Aquaculture in irrigated farming systems is also expanding in SE Asia (Haylor and Bhutta, 1997). These simplified ecosystems function with a small number of imposed fish species which may be of a known genetic quality (see above) in an empirically forced ecosystems with strong energetic flux and relatively few highly dominant prey species. They probably pilot the functioning of the system via their physiological potentiality, and as the system is operating on a short term basis, the biodiversity and its variability probably play a less important role. The integration of aquaculture in agrosystems is not only important in terms of biological efficiency but also to satisfy other needs for the farmers and societies such as landscape as reported by Ramakrishnan (1996).

#### *Association of several fish species for controlling overpopulation (concept of police fish)*

The introduction of piscivorous species in fish culture in pond is a common practice to get rid of the small unwanted fish, accidentally introduced or resulting from a precocious reproduction as in some tilapia species. One predator, the catfish *Clarias gariepinus*, was first used in Africa but the biomass to be introduced was high and it acted as a competitor for food in the pond with tilapias (*Oreochromis*). Such associations were recently refined by Lazard et Oswald (1995) in Africa with the use of a small cichlid, *Hemichromis fasciatus* which size does not exceed 30 g and eat fry of 3 g. Another police fish, *Parachanna obscura* was also introduced with success in the system. Attempts were made to use a catfish *Heterobranchus longifilis* as police fish but its growth rate was too high and it was feeding on growing-on tilapia and other police fish stocked fingerlings. Sometimes other species are associated with *Oreochromis* to increase the yield in quantity and quality, for example *Heterobranchus isopterus*.

#### **The use of aquatic and terrestrial plants as food for fish (concept of fish-crop integration)**

Aquaculture takes also advantage of the diversity of food, especially plants growing on the edge of the ponds. These systems are deeply integrated with agriculture and represent an efficient way of recycling matters (integrated fish farming). Management of plants for fish-crop integration has been described in China by Yang *et al.* (1992). They identified 12 different plants produced on pond dikes and drained pond bottoms (Table III). Plants were made available during the entire growing season. The yield was in the range of 17 to 50 kg of plant (wet weight) to produce one kg of live fish.

### The use of wastes (concept of fish-livestock integration)

The recycling of wastes in ponds to serve as food or fertilizers for fish represents another form of integration of aquaculture to agriculture. This approach is traditional in Asia, especially in China and is now also applied in Europe and in Africa where exotic carps have been introduced (Moreau and Costa-Pierce, 1997). The amount of organic matter which can be recycled in ponds as fertilisers is very high (up to 5 g C/m<sup>2</sup>/day corresponding to 100 kg of dry manure/ha/day). The expected production in a polyculture system (at least common carp and silver carp) may reach 30 kg/ha/day without adding exogenous food (Olah, 1986). The combination of polyculture with fish-livestock integration is very efficient in term of fish productivity (Table IV). Integration of algae culture with fish farming was attempted in southern Chili: *Gracilaria chilensis*, was experimentally cultivated on ropes near a salmon cage farm and could remove at least respectively 5 and 27% of dissolved nitrogen and phosphorous released from the farm (Troell *et al.*, 1997).

Table III. - Management of aquatic and terrestrial plants used for fish-crop integration (after Yang *et al.*, 1992).

Item	Growth period	Yield fresh (T/ha)	Food conversion factor (fresh)
Rye grass	Nov-May	75-150	17-23
Sudan grass	Jun-Oct	150-225	19-28
Elephant grass	Apr-Oct	225-450	30-40
Hybrid grass	Jun-Oct	225-300	25-30
Alfalfa	Apr-Oct	45-90	25-30
Clover	Apr-Oct	60-90	25-30
Indian lettuce	May-Oct	75-112.5	30-35
Chinese cabbage	all year	30-40 per crop	35-40
Cabbage	all year	30-40 per crop	35-40
Soybean	Jun-Oct	0.75-1.95	3
Water hyacinth	Jun-Oct	150-300	45-50
Water lettuce	Jun-Oct	150-225	45-50

Table IV. - Fish production in ponds, manured or not, stocked with different species (polyculture) (from Billard, 1991). Productions are given in kg/ha/year.

Species cultivated	Prey	Organic fertilization	No fertilization
Percb, .....	fish	170	13
Common carp, <i>Cyprinus carpio</i> , and Black carp, <i>Mylopharyngodon piceus</i>	zoobenthos and zooplankton	910	34
Grass carp, <i>Ctenopharyngodon idela</i>	macrophytes	263	7
Big head carp, <i>Aristichthys nobilis</i>	zooplankton	736	25
Mullet, .....	zooplankton	2502	17
Silver carp, <i>Hypophthalmichthys molitrix</i>	microzooplankton and phytoplankton	2706	262
Total		7287 kg	424 kg

### The impacts of fish culture in ponds on biodiversity

The impacts of fish culture on biodiversity depends mainly on the type of culture practiced. In polyculture combined with manuring, the impacts are rather positive on the environment as the release of waste material in the wild is limited but negative impacts on biodiversity may result from introduction of exotic genetic material. This includes highly domesticated species which can still reproduce in the wild and "hybridize" with local populations (Padhi and Mandal, 1994). The introduction of fish species in freshwater is a common practice and has been widely debated recently (Levêque, 1996). It is agreed that any introduction presents some risks and that they should be evaluated according to formal protocols.

Aquaculture may play a positive role in the conservation of biodiversity via the use of rearing technologies for propagation and stocking of endangered species. Philipart (1990) has listed more than 40 endangered freshwater fish species in Europe which are presently reared for conservation and stocking purposes. Biotechnologies used in "industrial" aquaculture may also serve as conservation tools for cultivated species such as gene banks including for instance Yangtze carp sperm (Harvey, 1996). Chromosome manipulations may be applied to *ex situ* conservation of endangered fish species. Kopeika (pers. comm.) has recently pointed out the quasi total extinction of the population of *Acipenser nudipectus* living in the Aral Sea; there is no female anymore and only few remaining males are stored in one hatchery. One possibility to "reconstitute" this population would be diploid androgenesis. Such technology was successfully used in acipenserids by Grunina *et al.* (1997); eggs of *A. baeri* and *A. stellatus* were irradiated (X Rays, 20 KR, in order to destroy the nuclear DNA) and were inseminated with a known number of spermatozoa resulting in dispermy. A heat shock (37°C, 2.5-3 min) induced diploidisation and further development of viable larvae.

### CONCLUSION

Fish culture especially in manured ponds makes use of the biodiversity by association of several species which feed at different levels of the trophic web and on a large diversity of additional exogenous food introduced in the system. In some cases piscivorous (police) fish are added to control overpopulation due to precocious reproduction or accidental introduction of small fish from the wild. Presently pond fish culture systems, largely based on polyculture, and the use of biodiversity yield more than 9 millions tons of fish for human consumption in a context of sustainable development without major negative effects on the environment and biodiversity. These systems of production are well adapted to produce food, particularly proteins, for local people in poor countries and allow the culture of fish without the use of expensive pelleted feeds (partly based on fish meal). Polyculture is a way to produce fish to compensate for the decline of capture fisheries and to satisfy the increasing demand of fish products. However polyculture is not very popular in rich countries because the fish produced have not a high commercial value (they are bony with sometimes mud taste) and a large research effort is now needed to process the fish according to the consumer requirements. Another way is to find new species of higher commercial values to be associated in polyculture systems. One example is now given by *Polyodon spathula* introduced in Central Europe which feed on phytoplankton and microzooplankton and is expected to produce good quality flesh and caviar.

The pond aquaculture systems operate under a high energetic flux on a short term basis and with a limited number of species operating in the trophic network and yielding high productivity. The genetic structure and variability of the fish compartment are relatively well known and they have been adapted to this environment over generations of fish farmers so that the biodiversity plays probably a less important role. The functioning of the prey compartment, mostly few species of bacteria and plankton, is probably more dependant on the physiological characteristic of the species (capacity of development in a highly eutrophic environment) than the biodiversity and its variability. But when the trophic chain was initiated a wider ecosystemic variability was available in the environment from which one combination of species (the most efficient ones in term of mineralisation of dead organic matter and growth ?) was selected and developed in the pond.

**Acknowledgements.** - This manuscript was typed by J. Faurillon. The review is part of a work supported by a EC INCO-COPERNICUS Grant (IC 15 CT 96-1005).

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## THE EFFECTS OF ACCLIMATION TEMPERATURE ON THE GROWTH PERFORMANCE AND THE ACTIVITIES OF ASPARTATE AND ALANINE AMINOTRANSFERASE OF THE AIR-BREATHING TELEOST, *CHANNA STRIATA* (CHANNIDAE)

by

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**ABSTRACT.** - *Channa striata*, collected in December (winter), February and May (summer), were exposed to three experimental temperatures, i.e., 32°C, 21°C and 16°C, respectively. The experimental groups along with the corresponding control fish were maintained in laboratory for 30 days and were fed with a diet containing 45% protein to find out the effect of temperature on the growth performance and the activities of aspartate (AsAT) and alanine (AlAT) aminotransferase. The enzyme activities were studied in liver, white muscle and serum. The food consumption was found to decrease with the decrease in water temperature in both control and experimental fish. Fish growth was found to be higher in the February group (control 2) and at an experimental temperature of 21°C with highest protein and feed efficiency. The AsAT activity was found to be higher than that of AlAT in the tissues studied in both experimental and control fish at all temperatures. Except the activity of AlAT in the liver of control fish, which remained almost the same, the aminotransferases in liver and serum of both experimental and control fish were found to increase with the increase in water temperature. The activity of AsAT in muscle increased significantly with the increase of the experimental temperature, from 21°C to 32°C, when the activity of AlAT remained unchanged. In the control groups the highest activity of muscle AsAT was observed in winter-adapted fish when the AlAT activity was found to be the same for summer- and winter-adapted fish.

**RÉSUMÉ.** - Les effets de la température d'acclimation sur la croissance et l'activité de l'aspartate et de l'alanine aminotransférases chez le téléostéen à respiration aérienne, *Channa striata* (Channidae).

Des spécimens de *Channa striata* récoltés en décembre (hiver), février et mai (été), furent exposés à trois températures expérimentales, i.e., 32°C, 21°C et 16°C. Les groupes expérimentaux et les groupes témoins furent maintenus au laboratoire pendant 30 jours et nourris avec des rations contenant 45% de protéines, afin de déterminer les effets de la température sur la croissance et l'activité de l'aspartate (AsAT) et de l'alanine aminotransférase (AlAT). L'activité des enzymes fut mesurée dans le foie, les muscles blancs et le sérum. La consommation de nourriture diminuait avec la baisse de température dans tous les groupes, expérimentaux et témoins. La croissance des poissons était plus forte en février (contrôle 2) et à la température d'expérience de 21°C, correspondant à la plus grande efficacité des protéines. L'activité de l'AsAT était plus grande que celle de l'AlAT dans les tissus étudiés pour tous les groupes, expérimentaux et témoins, et à toutes les températures. Alors que l'activité de l'AlAT restait constante dans le foie des groupes témoins, celle des aminotransférases augmentait avec la température d'expérience dans le foie et le sérum des groupes expérimentaux et témoins. L'activité de l'AsAT dans le muscle augmentait significativement avec les températures expérimentales de 21°C et 32°C, alors que l'activité de l'AlAT restait constante. Dans les groupes témoins, la plus grande activité de l'AsAT dans le muscle fut observée chez les poissons adaptés aux températures hivernales, alors que l'activité de l'AlAT restait constante chez les poissons adaptés aux températures hivernales et estivales.

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Key-words. - Channidae, *Channa striata*, Temperature, Aspartate, Alanine, Aminotransferase.

Aminotransferases play a major role not only in the deamination of amino acids but also in the formation of glucose through the process of gluconeogenesis, the later being an important pathway in the carnivorous fish given a normal diet (Cowey and Walton, 1989). Aspartate and alanine aminotransferases, the most important quantitative transaminases, has been widely studied in fish and detected in most fish tissue. The levels of these enzymes have been studied for a number of fish in relation to the protein content of the diet (Alexis and Papaparaskeva-Papoutsoglou, 1986). The relative levels of activities of these two enzymes in fish seem to depend on their nutritional status. In most teleost, the enzymes which initiate amino acid catabolism have been reported either to increase or to remain unchanged in relation to the protein content of diet. Serum and tissue levels of transaminases have been investigated in a number of teleosts and lungfishes, quite often under varied environmental conditions (Creach, 1967; Johnson, 1967; Bell, 1968).

Water temperature affects food intake, rate of transit of foodstuffs through the digestive tract, metabolic rate, etc. Temperature is also known to affect endogenous nitrogen excretion (Jobling, 1981). However, under well-fed conditions, the nitrogenous catabolic wastes, expressed as a proportion of nitrogen intake, are little affected by water temperature (Kaushik, 1981). During adaptation to a sudden rise in temperature, the nitrogenous losses are high. But once acclimatized, an increase in water temperature seems to affect only the voluntary food intake. A rise in water temperature appears then to affect nitrogen excretion and consequently overall nitrogen retention, only through its effect on food intake (Kaushik and Cowey, 1991). The effect of temperature and protein level on growth and protein gain of *Channa striata* were studied by Hogendoorn *et al.* (1983) and Degani *et al.* (1989). The purpose of this study is to examine the effect of temperature on the aspartate and alanine aminotransferases in liver, white muscle and serum of the carnivorous fish *Channa striata*, along with the growth patterns.

## MATERIAL AND METHODS

Hatchery reared fish of initial average weight 10 g (SD  $\pm$  2.2 g) were used for this experiment at three environmental temperatures, i.e., December (winter) (10-18°C), February (20-28°C), and May (summer) (25-35°C). Triplicate groups of winter and summer fish were acclimated to 32°C and 16°C, respectively, and three groups of fish collected in February were exposed to 21°C. Three other groups of fish, corresponding to each experimental temperature, kept at natural temperatures, were used as control. To avoid temperature shock, the experimental fish were gradually brought to the experimental temperatures and acclimated for ten days at those temperatures before beginning the experiment. The temperature was regulated by thermostatically regulated water heater or cold rooms as described by Das and Das (1985) to get the desired temperature ( $\pm$  1.5°C). The experimental and the control fish were maintained in 50 l fibre reinforced plastic tanks at a stocking rate of six fish per tank and fed with a formulated diet using brown fish meal as the major source of protein (Table I). All the dry ingredients were ground and mixed well along with the oils in an electrical grinder and the food was prepared as described previously (Samantaray and Mohanty, 1997). Fish were fed by hand as long as they were actively



accepting the food, two times a day, at 9:00 and 16:00 h, seven days a week for thirty days. The culture tanks were aerated from 9:00 to 16:00 h which was found optimum for this fish (Samantaray and Mohanty, 1997). The fish were acclimated to the formulated diet for a week at room temperature before beginning the temperature acclimation. The water of the culture tanks was changed partially on every alternate day and fully in every 15 days with the water previously maintained at the desired temperature.

At the beginning of the experiment the proximate composition of fish was recorded following methods described in AOAC (1975) to know the initial values. At the end of each treatment both the control and experimental fish were anaesthetized by MS-222 and blood was collected from the heart for enzyme assay and livers were removed for hepato-somatic index and enzyme assay. A small piece of dorsal muscle were removed for enzyme assay. The rest of the fish was dried to determine the moisture and other constituents as per AOAC (1975). Body protein content was determined based on Kjeldahl nitrogen (crude protein =  $N_2 \times 6.25$ ). Samples were extracted with petroleum ether for 8 h in Soxhlet extraction apparatus for crude fat determination. Ash content was estimated by incinerating the sample in a muffle furnace for 6 h at 550°C. Moisture was measured by oven-drying at 100°C to constant weight.

The blood was centrifuged at  $700 \times g$  to get the serum. The serum was stored at 4°C before the enzyme assay. Tissue homogenates (7.5% for muscle and 5% for liver) prepared in ice-cold 0.25 M sucrose (in 0.05 M potassium phosphate buffer, pH 7.5) at 4°C as described previously (Das *et al.*, 1986). The homogenate was centrifuged at  $15,000 \times g$  for 3 min at 4°C. The supernatant was decanted and kept at 4°C until the enzymes assays were completed.

The activity of aspartate aminotransferase (E.C. 2.6.1.1)(AsAT) and alanine aminotransferase (E.C. 2.6.1.2)(AlAT) was determined by the methods of Karmen (1955) and Wroblewski and La Deu (1956), respectively, at 25°C. The enzyme activities were ex-

Ingredient	% of weight
Fish meal <sup>1</sup>	32
Rice bran	17
Groundnut-oil cake	32
$\alpha$ -Cellulose	13
Carboxymethyl cellulose	1
Vegetable oil	2
Cod-liver oil	1
Vitamine <sup>2</sup> and mineral <sup>3</sup> mixture	2
<b>Nutrient content</b>	
Moisture (%)	8.5
Crude protein (%)	44.4
Crude lipid (%)	12.9
Ash (%)	10.0
Digestible energy <sup>4</sup> (kcal / 100 g)	432.0
Protein to digestible energy ratio (mg protein / kcal)	104.1

Table I. - Composition of experimental diet (% of weight) for *Channa striata*.

<sup>1</sup> Crude protein = 88.6%; crude lipid = 2.4%.

<sup>2</sup> To supply per 100 g diet: Vitamin A (as acetate), 10000 IU; cholecalciferol, 1000 IU; thiamin mononitrate, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 60 mg; cyanocobalamin, 30  $\mu$ g; nicotinamide 200 mg; ascorbic acid, 300 mg;  $\alpha$ -tocopheryl acetate, 50 mg; biotin, 0.5 mg.

<sup>3</sup> To supply per 100 g diet: calcium phosphate, 258 mg; magnesium oxide, 120 mg; ferrous sulphate, 64.08 mg; manganese sulphate, 4.06 mg; copper sulphate, 6.78 mg; zinc sulphate, 4.4 mg; sodium molybdate, 0.5 mg; sodium borate 1.76 mg.

<sup>4</sup> Calculated as 4.4 and 9 kcal/g for protein, carbohydrate and lipid respectively (Samantaray and Mohanty, 1997).

pressed as specific activities in units ( $\mu$ moles of DPNH oxidized / min) per mg protein for liver and muscle and as total activity in units / 100 ml of serum. Tissue proteins were determined by the method of Lowrey *et al.* (1951). The averages of the enzyme activities of experimental and control groups were compared by using student t-test (Youden, 1951).

## RESULTS

The food consumption was found to decrease with the decrease in water temperature. The growth, food conversion ratio (FCR) and protein efficiency ratio (PER) were found to be highest at a moderate temperature in February among the control groups and at 21°C among the experimental groups (Table II). The hepatosomatic index (HSI) (liver to whole body weight ratio) was almost similar in all the groups. Body protein content was found to increase with the decrease in water temperature among the control groups (Table III), but the same was found to be higher at 21°C among the experimental groups.

Table II. - Food efficiency, growth performance and hepatosomatic index in fingerling *Channa striata* fed a diet containing 45% protein and 432 kcal energy for 30 days at different natural and experimental temperatures (Mean  $\pm$  SE). <sup>1</sup> Protein efficiency ratio = weight gain in g / dry protein fed in g; <sup>2</sup> Food conversion ratio = dry feed fed in g / wet weight gain in g; <sup>3</sup> Hepato-somatic index = weight of liver in g / whole body weight in g.

Parameters	Control			Experimental		
	December 10-18°C	February 20-28°C	May 25-35°C	32° C	21° C	16° C
Initial weight (g)	10.80 $\pm$ 1.24	10.60 $\pm$ 1.20	10.80 $\pm$ 1.18	10.80 $\pm$ 1.10	10.80 $\pm$ 1.3	10.70 $\pm$ 1.30
Final weight (g)	16.20 $\pm$ 1.60	16.80 $\pm$ 2.00	15.50 $\pm$ 1.38	15.10 $\pm$ 1.33	15.80 $\pm$ 1.4	14.60 $\pm$ 1.30
Weight gain (%)	50.00 $\pm$ 3.43	58.40 $\pm$ 5.10	43.50 $\pm$ 5.64	39.80 $\pm$ 6.10	46.30 $\pm$ 8.4	36.50 $\pm$ 7.13
Mean daily feed consumption (% of body weight)	3.12 $\pm$ 1.10	3.56 $\pm$ 0.88	4.63 $\pm$ 1.13	4.47 $\pm$ 1.06	3.48 $\pm$ 1.0	2.94 $\pm$ 0.92
P E R <sup>1</sup>	0.73 $\pm$ 0.03	0.87 $\pm$ 0.01	0.52 $\pm$ 0.01	0.47 $\pm$ 0.02	0.66 $\pm$ 0.01	0.56 $\pm$ 0.01
F C R <sup>2</sup>	2.9 $\pm$ 0.04	2.3 $\pm$ 0.06	2.8 $\pm$ 0.03	3.8 $\pm$ 0.04	2.8 $\pm$ 0.08	2.9 $\pm$ 0.03
H S I <sup>3</sup>	1.16 $\pm$ 0.02	1.28 $\pm$ 0.01	1.26 $\pm$ 0.01	1.22 $\pm$ 0.03	1.24 $\pm$ 0.02	1.20 $\pm$ 0.01

Table III. - Body composition of *Channa striata* at different natural and experimental temperatures expressed as % wet weight basis (Mean  $\pm$  SE).

	Initial	Control			Experimental		
		December 10-18°C	February 20-28°C	May 25-35°C	32°C	21°C	16°C
Moisture	75.6 $\pm$ 2.9	73.4 $\pm$ 1.1	74.7 $\pm$ 1.3	76.2 $\pm$ 1.8	74.6 $\pm$ 1.8	73.8 $\pm$ 1.6	73.3 $\pm$ 1.8
Protein	15.8 $\pm$ 1.1	19.8 $\pm$ 0.8	17.3 $\pm$ 1.0	16.9 $\pm$ 0.9	17.0 $\pm$ 0.6	18.6 $\pm$ 1.0	17.9 $\pm$ 0.7
Fat	1.9 $\pm$ 0.5	2.9 $\pm$ 0.3	3.6 $\pm$ 0.8	2.2 $\pm$ 0.6	2.7 $\pm$ 0.7	3.2 $\pm$ 0.5	3.8 $\pm$ 0.6
Ash	4.9 $\pm$ 0.8	3.1 $\pm$ 0.5	3.9 $\pm$ 0.6	4.3 $\pm$ 0.8	5.3 $\pm$ 0.8	4.9 $\pm$ 0.3	3.7 $\pm$ 0.4

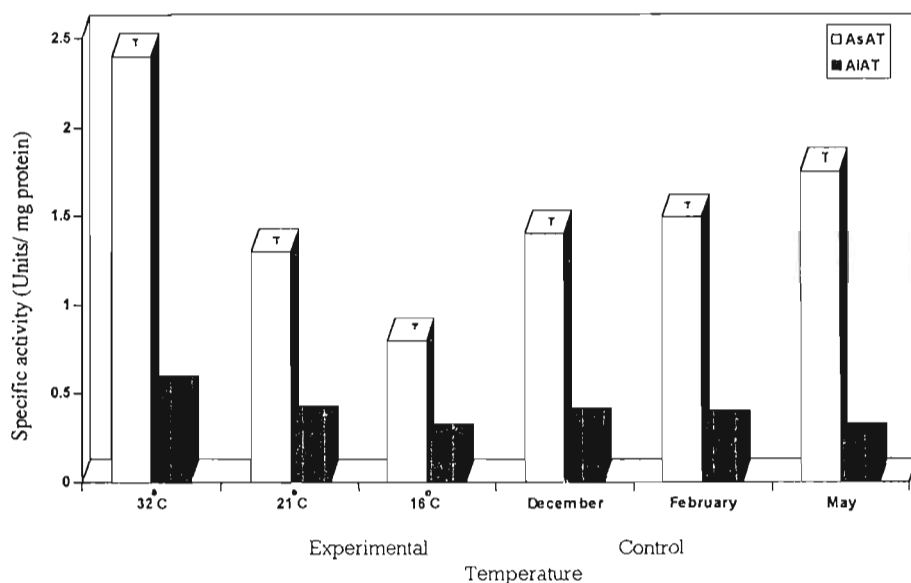


Fig. 1. - Specific activity of AsAT and AIAT in liver of fingerling *Channa striata* at different experimental and natural temperature (Mean  $\pm$  SE).

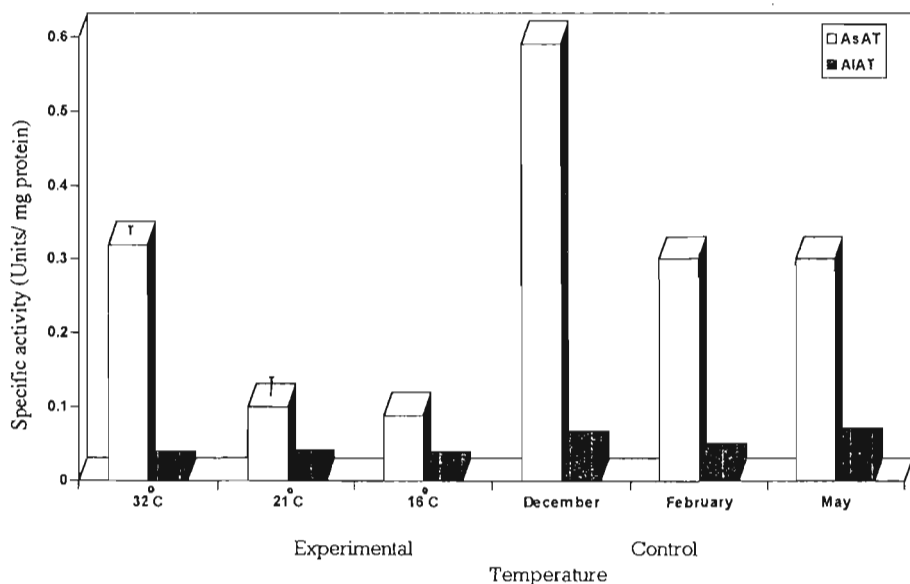


Fig. 2. - Specific activity of AsAT and AIAT in muscle of fingerling *Channa striata* at different experimental and natural temperature (Mean  $\pm$  SE).

On the other hand, the body fat content increase with the decrease in experimental temperature but highest body fat was observed in February (Control 2) among the control groups. Ash content was found to increase with the increase in temperature in both con-

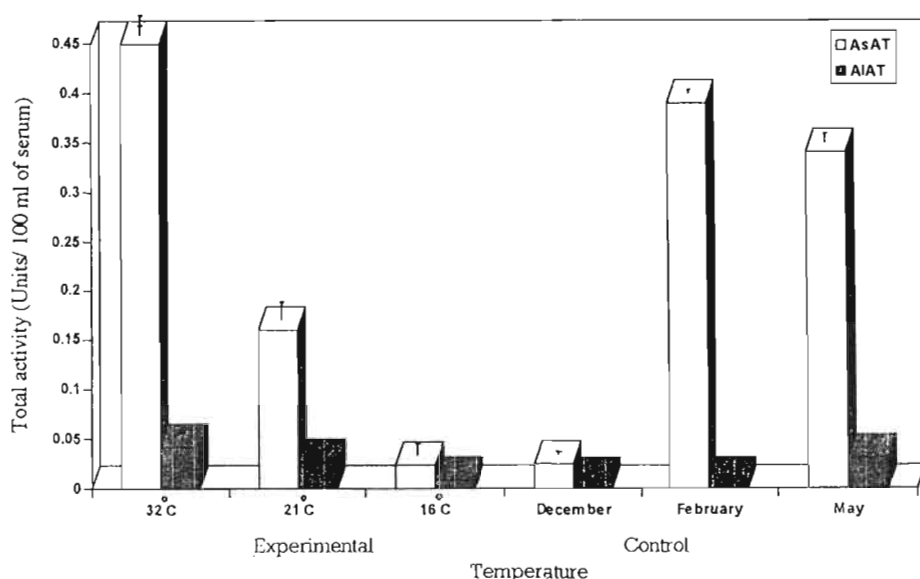


Fig. 3. - Total activity of AsAT and AIAT in serum of *Channa striata* at different experimental and natural temperature (Mean  $\pm$  SE).

trol and experimental fishes while the moisture content was not found to be affected by the temperature.

The changes in the aminotransferase activities of liver in the experimental and control groups were shown in figure 1. The AsAT activity increased at a significantly faster rate in both groups with the increase in water temperature than the AIAT. When the AIAT activity remained almost at the same level in all the control groups, it was found to increase significantly at the higher experimental temperature.

The aminotransferase activities of muscle (Fig. 2) are of smaller magnitude than that of liver. When the AsAT activity was found to increase gradually from lower to higher experimental temperature, significantly highest activity was observed in the winter-adapted control group when the activities in other two control groups were found to be almost same. No significant change was observed in the AIAT activity at the three experimental temperatures but the highest activity was observed in summer-adapted control with almost a similar value for the winter-adapted group.

Significant changes were observed in the total activity of the aminotransferase in blood serum of both experimental and control groups of *C. striata* (Fig. 3). In both groups the activities increase significantly from lower to higher temperature except that of AIAT in the control group at lower temperature.

## DISCUSSION

In general, temperature was not found to affect the optimum dietary protein requirement of fish (Cho *et al.*, 1985; Tacon and Cowey, 1985). Thus, a dietary protein level, found to be optimum for *Channa striata* (Samantaray and Mohanty, 1997), was used for this experiment at different temperature regimes. A direct effect of temperature was

observed in the present study on the growth performance (Table II) and proximate composition (Table III). However, it is not possible to discriminate the effect of water temperature on growth performance from other related factors such as fish size, feeding strategies, etc.

The growth rate and protein efficiency were observed to be highest at moderate experimental (21°C) and natural (February) temperatures and reverse at highest temperatures. Maximum values of metabolic rate (specific dynamic action or SDA) were reported around 25°C for mullet and linear negative relationship between SDA and temperature were found for temperatures above 25°C (Guinea and Fernandez, 1991). Since maintenance requirements are higher at high temperatures, allocation of higher proportions of energy to maintenance metabolism is a fact as the temperature rises. Thus, the proportion of the fixed ration, that can be directed to SDA would decrease with the increase in temperature, affecting the net protein synthesis (Guinea and Fernandez, 1991). This could be the reason for lower weight gain and protein efficiency at higher temperatures of this experiment. The higher food intake at higher temperature could not even found to bring any improvement in the weight gain. Cold temperature inhibits feeding and reduces protein synthesis to relatively lower level due to some plasticity in contractile properties in muscle (Heap *et al.*, 1986). Lower food intake and protein efficiency observed in our experiments at lower temperatures (16°C and December) could be due to the same reason. Even though lower daily protein intake was reported for sea bass (*Dicentrarchus labrax*) at 15°C than that of 20°C, the protein requirement for weight gain was higher at 15°C because of the lower gross conversion efficiency (Hidalgo and Alliot, 1988). However, in the present study the trend in daily protein intake level was similar to that of sea bass at similar experimental temperatures of 16°C and 21°C but the protein requirement was lower for weight gain at 16°C as the food efficiency is almost the same at both temperatures.

Within a given feeding regime, increased temperature lowered the hepato-somatic index (HSI) in largemouth bass (*Micropterus salmoides*) (Heidinger and Crawford, 1977). The influence of diet composition on HSI seemed to be highly significant in sea bass at 15°C and 20°C due to increase in liver size according to the digestible carbohydrate level in the diet (Hidalgo and Alliot, 1988) but in *Channa striata* no change in HSI was observed which may be due to the type of diet used.

When the body protein content (Table III) increased with the decrease in natural temperatures (control groups), no significant change was observed with the change in the experimental temperatures. On the other hand the fat accumulation was higher with the decrease in the experimental temperature but significantly higher body lipid content was noticed at a moderate natural temperature (February). Since maintenance requirements are more at high temperatures, neither protein nor fat accumulation was observed at those temperatures (32°C and May) which implies that higher energy diet is needed at high temperature. Hidalgo and Alliot (1988) have correlated the body protein and lipid content with diet at 15°C for sea bass stating the use of non-protein energy for the changes in carcass protein and lipid content.

AsAT activities were found to be remarkably higher in both the tissue and serum of both control and experimental fishes. Similar, but lesser degree of differences were observed in *Ictalurus punctatus* (Willson, 1973), *Cyprinus carpio* (Nemscok *et al.*, 1981) and *Channa punctatus* (Das *et al.*, 1986).

The different behaviour of liver enzymes of the fish species studied so far, with varied protein level in diet where attributed to the genetically determined capacity in utilizing protein as an energy source or to the level of energy available to the fish in the

diet (Alexis and Papaparaskeva-Papoutsoglou, 1986). In the carnivorous fish like *Channa*, the metabolism might be geared towards the use of more protein as an energy source, so that a portion of the ingested protein is deaminated to meet the energy need. In the present study the hepatic AsAT and AlAT activities (Fig. 1) was found to be influenced by the temperatures under both experimental and natural conditions. While studying the seasonal variation in the enzyme activities in *C. punctatus*, Samantaray *et al.* (1996) have revealed a gradual augmentation in the two aminotransferase activities in liver from late winter (February-March) resulting in a clear peak in summer (April-May). Aspartate in liver is generally involved in the synthesis of proteins, pyrimidines, purines and it would therefore be expected that liver tissue would require a fairly active system for a continuous synthesis of aspartate to meet high rate of protein catabolism at high temperature (Samantaray *et al.*, 1996).

The influence of temperature on the muscle aminotransferase level was found to be less significant (Fig. 2). The response of aminotransferases to temperature in the experimental and control groups were different. When the activity of both enzymes increases gradually with the increase in experimental temperature, the AsAT activity was found to be highest in winter (control 1) fish and that of AlAT was almost same in both summer (control 3) and winter temperature. In an annual cycle, the activity of AsAT in *C. punctatus* muscle tend to rise from early winter (October-November) to give a remarkable peak in winter (December-January) (Samantaray *et al.*, 1996). The presence of aspartate, glutamate, 2-oxoglutarate and oxaloacetate would provide a buffer level of oxaloacetate for the operation of TCA cycle and / or as shuttle in the transfer of reducing equivalents. It may be for this reason that muscle contains so much of this equilibrating enzyme. An additional function of AsAT in muscle could be to mediate a constant supply of aspartate for the conversion of inosinic acid to AMP.

Alanine aminotransferase is reported to play a greater role in non-essential amino acid metabolism in channel catfish (Wilson, 1973). The relative roles of AlAT and AsAT in gluconeogenesis of starving fish tissue are still debatable, although in mammals, the aminotransferase participating in gluconeogenesis during starvation is AlAT (Wittenberger and Giurgea, 1973). The lower food intake due to the decrease in the experimental temperature might be the cause of higher aminotransferase in muscle in the present study. The metabolism of the muscle tissue is different from that of the liver with branched chain amino acids; glutamine and alanine formation play an important role in protein synthesis and degradation (Goldberg and Chang, 1978). The biochemical mechanisms of the various processes are not clearly known.

The aminotransferase activities in serum was found to increase with the rise in temperature (Fig. 3). Similar temperature-dependent activities were observed in *C. punctatus* to give a clear peak in summer (May) with a gradual fall to the minimal levels in winter (December) in an annual cycle (Samantaray *et al.*, 1996). The presence of the enzyme in serum is due to the leakage from liver where the enzymes are synthesized. Thus, the fluctuations in the levels of enzymatic activities in the serum was found to be correlated with that of liver.

Since temperature is the most predominant environmental factor that affects the metabolic activities of fish, it is highly essential to conduct further studies on the activities of these enzymes at various protein-to-energy levels at different seasonal temperatures to find out the relative importance of various energy levels at different seasons for *C. striata*, for the development of low-cost diets.

**Acknowledgements.** - Financial assistance received from Indian Council of Agricultural Research, Government of India, for this work is gratefully acknowledged. Thanks are due to the Central Institute of Freshwater Aquaculture for providing facilities for this experiment.

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## Symposium 12

### REPRODUCTION, GROWTH, PHYSIOLOGY

Chairperson: **François MEUNIER**

- MARTIN-SMITH K. & L. LAIRD. - Reproductive patterns in some Cypriniformes from Borneo. 493-504

*Stratégies de reproduction chez quelques Cypriniformes de Bornéo.*

- YAMAGUCHI A., HUANG S.-Y., CHEN C.-T. & T. TANIUCHI. - Age and growth of the star-spotted smooth-hound, *Mustelus manazo* (Chondrichthyes: Triakidae) in the waters of north-eastern Taiwan. 505-513

*Âge et croissance de l'émissole étoilée, Mustelus manazo (Chondrichthyes: Triakidae), dans les eaux nord-orientales de Taiwan.*



## REPRODUCTIVE PATTERNS IN SOME CYPRINIFORMES FROM BORNEO

by

Keith MARTIN-SMITH (1, 2) & Lindsay LAIRD (1)

**ABSTRACT.** - In the tropics freshwater fishes are not necessarily constrained to seasonal reproduction by physical conditions. This study investigated the reproductive allocation of ten species of freshwater fishes from Sabah, East Malaysia to determine whether reproduction was seasonal and whether general patterns existed across species. Monthly ovary samples were taken from seven cyprinid species and three balitorid species and the gonado-somatic index (GSI) calculated. Potential fecundity was estimated for seven species. Two cyprinid species, *Garra borneensis* and *Lobocheilos bo*, showed unimodal, seasonal reproduction with peak GSI values in December-January, corresponding with the major rainy season, while *Puntius sealei* showed a similar peak in GSI but earlier in the year (in June-July). *Homaloptera stephensoni* showed an extended GSI peak from November-March while *Gastromyzon danumensis* showed two peaks in reproductive effort, in December and in April. The other cyprinid species, *Nematabramis everetti*, *Osteochilus chini*, *Rasbora hubbsi* and *R. sumatrana*, and the balitorid *Gastromyzon lepidogaster* all showed year-round gonad maturity. Populations of these latter five species showed no reproductive synchrony; randomly sampled individuals of the same size displayed large variations in their GSI. Peak GSI values for different species varied widely from 7.5-23.0%. These results indicate that different species pursue different life-history strategies in the allocation of reproductive effort.

**RÉSUMÉ.** - Stratégies de reproduction chez quelques Cypriniformes de Bornéo.

Sous les tropiques, les poissons d'eau douce ne sont pas nécessairement soumis à une reproduction saisonnière du fait des conditions physiques du milieu. Ce travail est une étude de la stratégie de reproduction de dix espèces de Cypriniformes de Sabah, Est-Malaisie, pour déterminer si la reproduction est saisonnière et si des modèles généraux existent chez ces espèces. Des prélèvements mensuels d'ovaires ont été faits sur sept Cyprinidae et trois Balitoridae et l'indice gonado-somatique (IGS) a été calculé. La fécondité potentielle a été estimée pour sept espèces. Deux Cyprinidae, *Garra borneensis* et *Lobocheilos bo*, ont montré une reproduction saisonnière unimodale avec un pic maximal des valeurs d'IGS pendant les mois de décembre-janvier, correspondant à la grande saison des pluies, tandis que *Puntius sealei* montrait un pic similaire, mais plus tôt dans l'année (en juin et juillet). *Homaloptera stephensoni* montrait un pic d'IGS prolongé de novembre à mars, tandis que *Gastromyzon danumensis* présentait deux pics d'effort reproductif, en décembre et en avril. Les autres espèces de Cyprinidae, *Nematabramis everetti*, *Osteochilus chini*, *Rasbora hubbsi* et *R. sumatrana*, et le Balitoridae *Gastromyzon lepidogaster*, ont des gonades matures tout au long de l'année. Les populations de ces cinq dernières espèces n'ont pas montré de synchronisation de la reproduction; un échantillon d'individus de même taille, prélevé au hasard, exhibait de grandes variations de leur IGS. Le pic des valeurs d'IGS pour les différentes espèces variait de 7.5 à 23%. Ces résultats indiquent que ces espèces développent des stratégies de reproduction différentes.

**Key-words.** - Cyprinidae, Balitoridae, Reproduction, Seasonality, Borneo.

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Reproductive seasonality is an essential element in life history strategies for fishes living in highly seasonal environments, allowing them to synchronise reproductive effort for maximum survival of young (see review by Bye, 1984). While the tropics are considered to be aseasonal with respect to variations in photoperiod and temperature, many regions show distinct seasonality with respect to rainfall or food availability. This seasonality has been shown to be correlated with reproductive effort or spawning in many tropical freshwater fishes (e.g., Welcomme, 1969; Sobhana and Balakrishnan Nair, 1974; Kramer, 1978; Harikumar *et al.*, 1994), although there are examples of year-round spawning in some species (Kramer, 1978). For many parts of the world, however, there are not enough data to be able to make generalisations about the patterns of reproduction.

This study investigated the natural patterns of reproductive allocation in a representative group of fishes from Sabah, east Malaysia, part of the island of Borneo. Nothing is known about reproductive seasonality in the vast majority of the 400 + species of freshwater fishes inhabiting Borneo, especially the smaller species living in headwater streams (Kottelat and Whitten, 1996). Although Sabah has seasonal patterns of rainfall influenced by edge effects of both the north-east and south-west monsoons, timing and amount of rainfall are highly variable (Walsh, 1996). Severe periodic droughts of up to 5 months are known to occur (Beaman *et al.*, 1985). If rainfall patterns act as a cue for reproduction, such variability may strongly influence reproductive strategies. Thus the explicit aims of this paper were to quantify the annual gonad cycles for ten species of cypriniform fishes from Sabah and to relate these patterns to climate, particularly rainfall.

## MATERIAL AND METHODS

### Study site and species

Females of ten species of fishes were sampled from three streams in the vicinity of Danum Valley Field Centre (DVFC), Sabah. These streams, Palum Tambun stream (PTA), a tributary of Bole Kecil River (BKT) and Lonpodas stream (LPO), are all tributaries of the Segama River, one of the two major drainage systems on the east coast of Sabah. A description and map of these sites is given in Martin-Smith (1998). Data on rainfall and temperature were recorded daily by the Manchester University DVFC Hydrology Project.

Table 1. - Characteristics of fish species sampled for reproductive seasonality in Sabah.

Species	Family	Habitat	Maximum SL (mm)	Sexual dimorphism	Sampling period
<i>Garra borneensis</i>	Cyprinidae	Run/Riffle	110	Yes	Oct. 95 - Feb. 97
<i>Gastromyzon danumensis</i>	Balitoridae	Riffle	41	No	Oct. 96 - Aug. 97
<i>Gastromyzon lepidogaster</i>	Balitoridae	Riffle	85	No	Oct. 96 - Aug. 97
<i>Homaloptera stephensoni</i>	Balitoridae	Riffle	70	Yes	Oct. 96 - Aug. 97
<i>Lobocheilus bo</i>	Cyprinidae	Run/Pool	> 250	Yes ?	Sep. 95 - Nov. 96
<i>Nematabramis everetti</i>	Cyprinidae	Pool	105	No	Sep. 95 - Jan. 97
<i>Osteochilus chini</i>	Cyprinidae	Pool	175	No	Sep. 96 - Jul. 97
<i>Puntius sealei</i>	Cyprinidae	Pool	110	No	Sep. 96 - Jul. 97
<i>Rasbora hubbsi</i>	Cyprinidae	Riffle	52	No	Sep. 96 - Aug. 97
<i>Rasbora sumatrana</i>	Cyprinidae	Pool	105	No	Sep. 95 - Jan. 97

Study species were selected on the basis of their ecological importance in local stream communities - all species were abundant and widespread (Martin-Smith, 1998). Seven species were from the Cyprinidae, four pool specialists, one riffle specialist and two ubiquitous, while three species were from the Balitoridae (hillstream loaches), all riffle specialists (Martin-Smith, 1998). Details of all species are given in table I.

### Sampling procedure and data analyses

Fish were sampled monthly over the period Sep. 95 - Aug. 97. For logistical reasons, not all species could be collected over the entire period. Each species was sampled for a minimum of 11 consecutive months, most species for > 12 months (Table I). An attempt was made to collect ten mature females from each species being studied from two sites each month. Preliminary collections of all species were undertaken to establish the size range of reproductive individuals and to establish whether there was any sexual dimorphism. Most species showed no obvious sexual dimorphism so all individuals of reproductive size were collected. An attempt was made in the field to sex individuals by gently squeezing the abdomen to expel milt or a few eggs, but this was not always effective. During some months fewer than ten mature females could be collected, usually due to difficulties associated with high water levels, flooding and high turbidity. Fishes were generally sampled by electrofishing, although some of the larger species were occasionally collected using a cast net (20 mm stretched mesh). All individuals were killed with an overdose of benzocaine solution and then fixed immediately in 10% formalin. In the laboratory the standard length (SL) and somatic weight ( $W_t$ ) of individuals were recorded and the ovaries excised and weighed ( $W_o$ ). Ovaries were then stored in 10% formalin. Total counts of mature oocytes were made for all samples from at least two different months for all species except *Garra borneensis*, *Lobocheilos bo* and *Osteochilus chini*.

Size-specific sexual dimorphism was tested for each species by comparing length-frequency distributions for the entire data set (including the preliminary collections) using Kolmogorov-Smirnov two-sample tests. For each individual fish the gonado-somatic index (GSI) was calculated as  $W_o / W_t \times 100$  and the condition factor (K) as  $W_t / \text{expected weight } (W_e)$ .  $W_e$  was calculated from the linear regression of  $\log(W_t)$  on  $\log(SL)$  for the total data set. Differences in mean GSI and K values between sites over all time points were tested by Spearman rank correlation ( $R_s$ ) since data were not normally distributed. Where sites were significantly correlated data were combined for further analysis. Differences in GSI and K values between months were tested using one-way analysis of variance (ANOVA) and significant differences between adjacent months identified using Fisher's PSLD test.

The relationship between the number of oocytes ( $N_o$ ) and measures of body size (length or weight) is frequently of the form  $N_o = aZ^b$  where  $a$  and  $b$  are constants and  $Z$  is the measure of body size (Bagenal and Braum, 1978). The relationship between  $N_o$  and  $W_t$ ,  $W_e$  and SL were therefore investigated using linear regression on log transformed data.

## RESULTS

Seasonal variation in climate is shown in figure 1. Temperature varied little throughout the sampling period, with less than 2°C difference in mean air temperature between months. Rainfall was more seasonal with long-term averages showing wet periods in Nov. - Feb. (north-east monsoon) and May - Jul. (south-west monsoon) with shorter dry periods in Mar. - Apr. and Aug. - Oct. During the period of the study rainfall

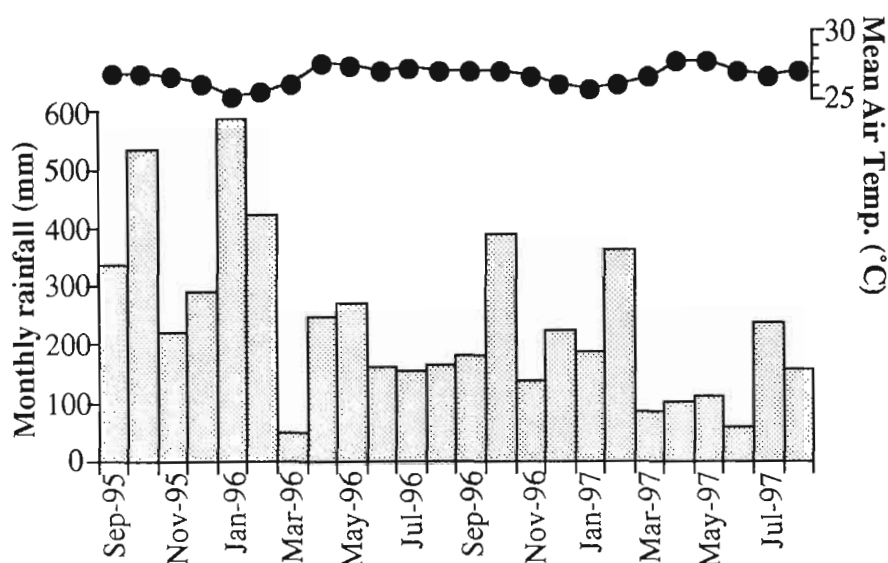


Fig. 1. - Climate records for DVFC (data courtesy of University of Manchester Hydrology Project). Monthly rainfall and mean air temperature.

was highest from Oct. 95 - Feb. 96, Apr. - May 96 and Oct. 96 - Feb. 97, while there was a prolonged drought from Mar. - Jun. 97 (Fig. 1).

Size-specific sexual dimorphism was widespread with mature females being significantly larger than mature males in *Gastromyzon lepidogaster*, *Homaloptera stephensoni*, *Lobocheilos bo*, *Osteochilus chini*, *Puntius sealei* and both species of *Rasbora* (Table II, Appendix I). Minimum size at first reproduction was estimated from the smallest mature female found during the entire sampling period and is given in table II.

For the months and species where differences between sampling locations could be tested there were few significant differences in mean GSI and condition, so samples were

Table II. - Reproductive parameters for ten species of fishes from Sabah. \*:  $p < 0.05$ , Kolmogorov-Smirnov two-sample test. †: too few males to test significance.

Species	Min. SL of mature female (mm)	Max. SL of mature female (mm)	Significant size difference between sexes*	Maximal GSI value (%)	Date of max. GSI	Number of oocytes
<i>Garra borneensis</i>	52	106	No	22.3	Feb. 97	-
<i>Gastromyzon danumensis</i>	24	38	No	17.0	Oct. 96	234-1441
<i>Gastromyzon lepidogaster</i>	48	77	Yes (F>M)	11.7	Mar. 97	174-1504
<i>Homaloptera stephensoni</i>	41	69	Yes (F>M)	18.3	Dec. 96	288-3154
<i>Lobocheilos bo</i>	86	204	? Yes (F>M)†	18.5	Nov. 96	-
<i>Nematabramis everetti</i>	59	95	No	7.5	Apr. 96	37-1556
<i>Osteochilus chini</i>	85	175	Yes (F>M)	13.9	Feb. 97	524-5880
<i>Puntius sealei</i>	58	102	Yes (F>M)	25.1	Sep. 96	54-4550
<i>Rasbora hubbsi</i>	35	53	Yes (F>M)	16.2	Mar. 97	80-2148
<i>Rasbora sumatrana</i>	65	97	Yes (F>M)	12.1	Feb. 96	84-3436

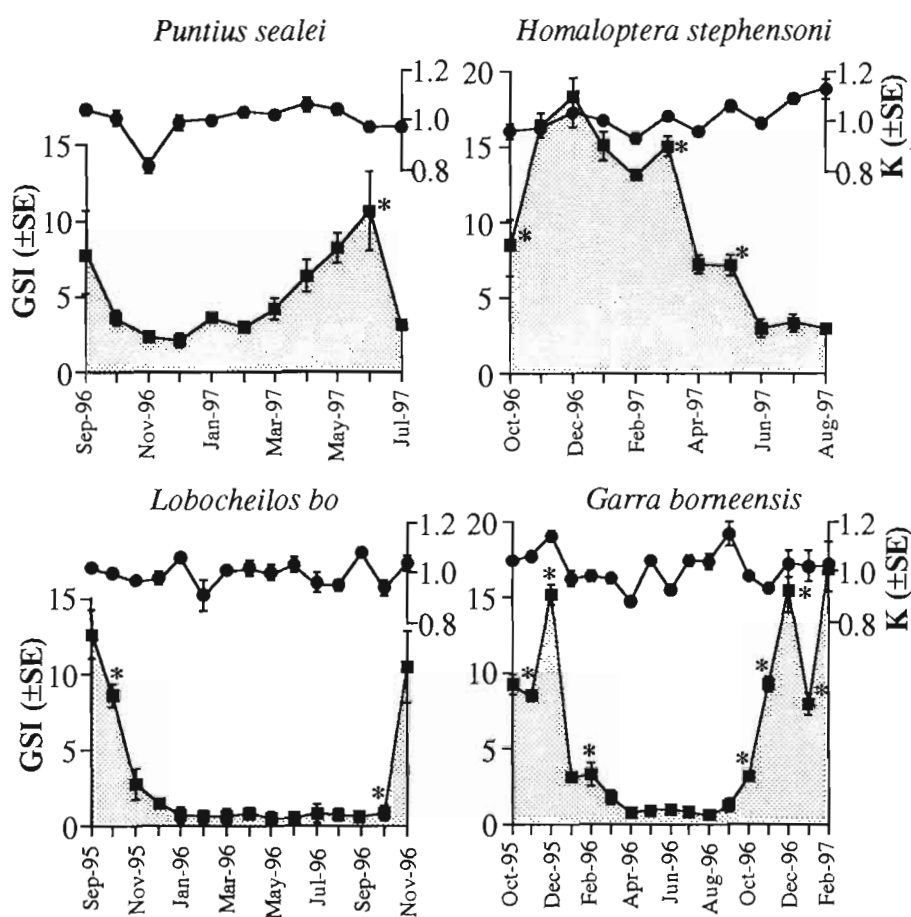


Fig. 2. - Mean monthly GSI (■) and condition (●) values for four species of fish displaying seasonal reproduction. \*: significantly different GSI value from subsequent months.

combined for further analysis. Five species showed seasonal patterns of GSI values (Fig. 2). Four of these, *Garra borneensis*, *H. stephensoni*, *L. bo* and *P. sealei*, showed unimodal peaks, while *Gastromyzon danumensis* had a bimodal pattern. *G. borneensis* and *L. bo* showed abrupt changes in GSI values with seasonal maxima in Nov. - Feb. for *G. borneensis* (mean GSI > 15%) and slightly earlier for *L. bo* (mean GSI > 12%). These corresponded with the start of the major rainy season. *Homaloptera stephensoni* had a similar pattern except the duration of the GSI peak was much longer (Nov. - Mar., mean GSI > 17%), extending for the entire duration of the major rainy season in 1996-1997. The GSI maximum for *P. sealei* occurred earlier in the year (Jun. - Sep., mean GSI > 11%) during a period of drought. *Gastromyzon danumensis* showed two peaks in mean GSI values, in Nov. (start of rainy season) and Apr. (drought) with mean GSI > 12%. However, the apparent drop in GSI for Dec. 96 - Feb. 97 may be an artifact of low sample numbers, disguising a pattern similar to that of *H. stephensoni*. Although there was some variability in individual GSI values, especially for *H. stephensoni* and *P.*

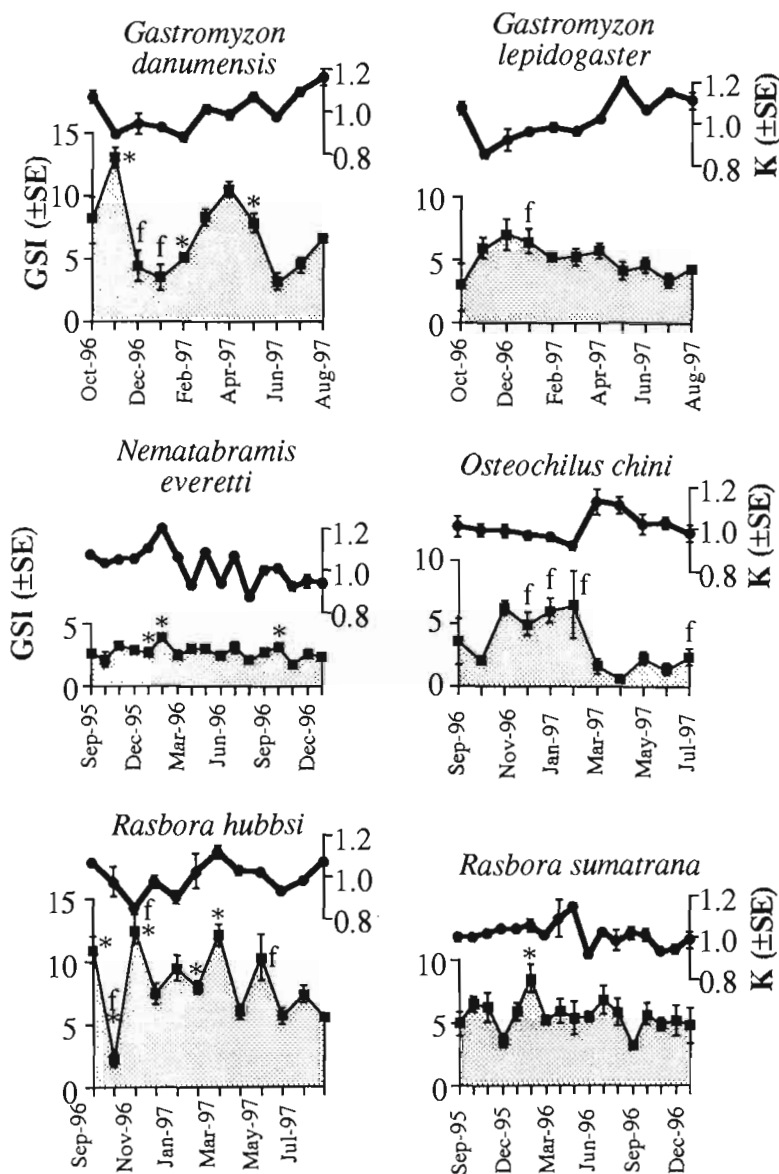


Fig. 3. - Mean monthly GSI (■) and condition (●) values for six species of fish displaying bimodal or aseasonal reproduction. \*: significantly different GSI value from subsequent months; f: less than five samples.

*sealei*, all species showing seasonal reproduction generally demonstrated within-population synchrony. Of the five species showing seasonal reproduction only *G. borneensis* and *H. stephensoni* showed an interpretable pattern of variation in mean K values (Figs 2, 3); in both cases increased K was correlated with a drop in GSI.



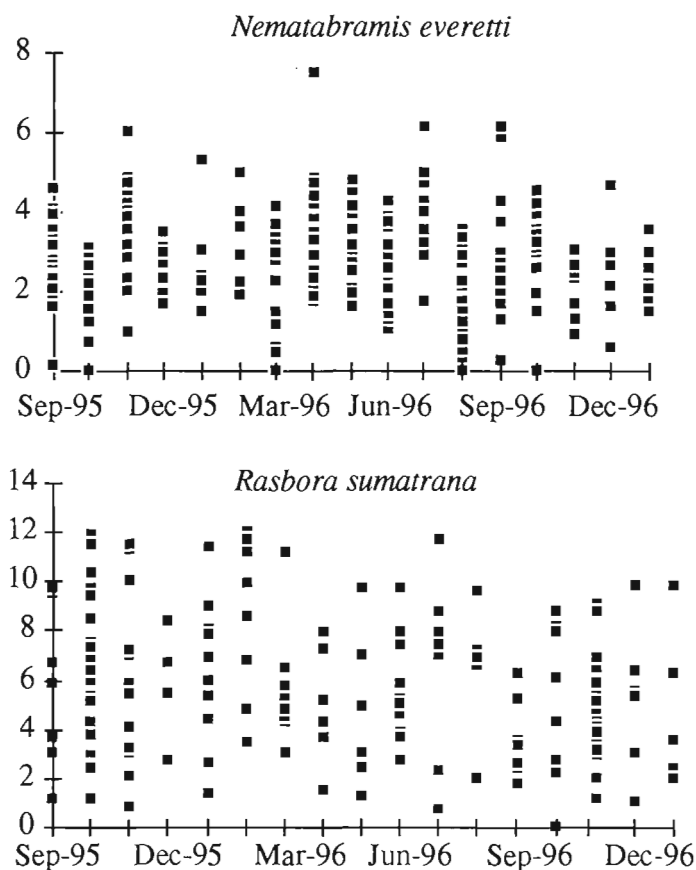


Fig. 4. - Individual GSI values for two species of fish displaying aseasonal reproduction.

The remaining five species, *Gastromyzon lepidogaster*, *Nematabramis everetti*, *Osteochilus chini*, *Rasbora hubbsi* and *R. sumatrana*, showed no clear patterns of GSI change over the sampling period (Fig. 3). There were no significant changes in mean GSI values between any adjacent pairs of months for *G. lepidogaster* and *N. everetti*. There

Table III. - Relationship between ovary weight and fecundity for seven species of fish from Sabah. \*\*: significant at  $0.05 < p < 0.001$ ; \*\*\*: significant at  $p < 0.001$ .

Species	n	$r^2$	$\log a \pm 95\% \text{ CI}$	$b \pm 95\% \text{ CI}$
<i>Gastromyzon danumensis</i>	19	0.47**	$3.201 \pm 0.239$	$0.405 \pm 0.221$
<i>Gastromyzon lepidogaster</i>	10	0.78***	$3.242 \pm 0.176$	$0.593 \pm 0.256$
<i>Hemaloptera stephensoni</i>	20	0.82***	$3.811 \pm 0.185$	$1.150 \pm 0.267$
<i>Nematabramis everetti</i>	32	0.74***	$3.666 \pm 0.229$	$1.629 \pm 0.360$
<i>Puntius sealei</i>	21	0.85***	$3.291 \pm 0.098$	$1.174 \pm 0.227$
<i>Rasbora hubbsi</i>	19	0.91***	$3.608 \pm 0.218$	$1.228 \pm 0.318$
<i>Rasbora sumatrana</i>	49	0.80***	$3.245 \pm 0.076$	$0.734 \pm 0.178$

were significant changes in mean GSI for *R. hubbsi* and *R. sumatrana* but the direction of these changes was often reversed in the following month. Finally, although *O. chini* showed a possible peak in GSI from Nov. 96 - Feb. 97, sample sizes were low in this species - despite intensive sampling few mature individuals could be found. Further evidence of the lack of seasonality in these five species was provided by examination of the spread of individual GSI values, which showed similar amplitude at all sampling times, examples of which are given in figure 4. Similarly, mean K values showed no easily interpretable patterns, except perhaps a negative correlation with GSI in *O. chini* (Fig. 3).

Counts of mature oocytes were strongly correlated with  $W_t$  for all species (Table III). Oocyte counts were correlated with SL for four species (Table IV) and  $W_t$  for six species (Table V). In all cases  $W_t$  was a better predictor of  $N_e$  than SL, but variability was still high, reflected in low regression coefficients.

## DISCUSSION

A number of patterns of reproductive seasonality were displayed by the fishes investigated, ranging from a restricted period of gonad maturation (*G. borneensis*, *G. danumensis* and *L. bo*) through extended GSI peaks (*H. stephensoni* and *P. sealei*) to aseasonality (other species). These patterns have been documented for some other tropical species, especially in Africa and South America (Kramer, 1978; Lowe-McConnell, 1987;

Table IV. - Relationship between standard length and fecundity for seven species of fish from Sabah. ns = not significant at  $p = 0.05$ ; \*\*: significant at  $0.05 < p < 0.001$ ; \*\*\*: significant at  $p < 0.001$ .

Species	n	$r^2$	$\log a \pm 95\% \text{ CI}$	$b \pm 95\% \text{ CI}$
<i>Gastromyzon danumensis</i>	19	0.38**	$-9.339 \pm 5.402$	$5.531 \pm 3.595$
<i>Gastromyzon lepidogaster</i>	10	0.04 <sup>ns</sup>	-	-
<i>Homaloptera stephensoni</i>	20	0.07 <sup>ns</sup>	-	-
<i>Nematabramis everetti</i>	32	0.03 <sup>ns</sup>	-	-
<i>Puntius sealei</i>	21	0.46***	$-7.626 \pm 3.638$	$3.860 \pm 1.907$
<i>Rasbora hubbsi</i>	19	0.34**	$-9.936 \pm 6.370$	$5.585 \pm 3.819$
<i>Rasbora sumatrana</i>	49	0.13**	$-7.820 \pm 5.502$	$3.952 \pm 2.895$

Table V. - Relationship between somatic weight and fecundity for seven species of fish from Sabah. ns = not significant at  $p = 0.05$ ; \*\*: significant at  $0.05 < p < 0.001$ ; \*\*\*: significant at  $p < 0.001$ .

Species	n	$r^2$	$\log a \pm 95\% \text{ CI}$	$b \pm 95\% \text{ CI}$
<i>Gastromyzon danumensis</i>	19	0.42**	$-1.229 \pm 0.168$	$1.856 \pm 1.048$
<i>Gastromyzon lepidogaster</i>	10	0.09 <sup>ns</sup>	-	-
<i>Homaloptera stephensoni</i>	20	0.40**	$-1.064 \pm 0.268$	$1.148 \pm 0.690$
<i>Nematabramis everetti</i>	32	0.28**	$-1.773 \pm 1.069$	$0.968 \pm 1.033$
<i>Puntius sealei</i>	21	0.54***	$-2.135 \pm 0.793$	$1.525 \pm 0.640$
<i>Rasbora hubbsi</i>	19	0.48***	$-1.351 \pm 0.386$	$1.878 \pm 0.963$
<i>Rasbora sumatrana</i>	49	0.24***	$-2.169 \pm 0.928$	$1.840 \pm 0.915$

Winemiller, 1989). Few studies appear to have been carried out on southeastern Asian species (Abidin, 1986; Khan *et al.*, 1990). The only comparable study on southeastern Asian cyprinids found an extended GSI peak for 7 months in *Hampala macrolepidota* in peninsular Malaysia (Abidin, 1986). No data have been found on reproductive seasonality in balitorids.

A number of hypotheses have been advanced as to the adaptive advantages of different reproductive strategies (Kramer, 1978; Lowe-McConnell, 1987; Winemiller, 1989). Spawning at the beginning of the wet season coincides with peak food availability in floodplain areas (Lowe-McConnell, 1987), while continuous spawning throughout the year can be seen to be an "opportunistic" strategy to take advantage of favourable biotic or abiotic conditions (Winemiller, 1989). Competition for spawning sites or decreased predation on vulnerable early life stages are other hypotheses which may favour seasonal reproduction. There are no data on spawning locations or predators of eggs and fry in Borneo. Finally, « phylogenetic inertia » (Kramer, 1978) may constrain fishes to seasonal reproduction when there is no adaptive advantage. This may be the case for *Puntius* - Sobhana and Balakrishnan Nair (1974) found peak GSI values in June and September in *P. sarana subnasutus* similar to *P. sealei* in the present study despite large differences in climatic regime. In contrast, De Silva (1991) found that *Garra ceylonensis* bred after the rainy season in Sri Lanka rather than at the beginning as in *G. borneensis*.

Seasonal reproduction is often correlated with the pattern of egg release. Eggs may be released at the same time (total spawners) or in batches (partial and small-brood spawners). Detailed histological investigations have been carried out on *Garra borneensis* and *Nematabramis everetti* which show that the former is a total spawner while the latter is a small-brood spawner (Maybank, 1996; Martin-Smith *et al.*, unpubl. data). It is suggested that both *Rasbora* species and *Gastromyzon lepidogaster* are also small-brood spawners and that *Lobocheilos bo* is a total spawner. *Homaloptera stephensoni* has a GSI pattern characteristic of a partial spawner i.e., extended reproductive season (Lowe-McConnell, 1987) and histological analysis has confirmed the presence of multiple batches of maturing oocytes (McCaig, 1998).

It is possible that the differences in timing and mode of reproduction are related to ecological factors such as habitat or food source. Of particular interest are the differences and similarities between congeneric pairs of species. *Gastromyzon danumensis* and *G. lepidogaster* had different patterns of GSI values through the year, yet inhabit similar microhabitats and usually occur together (Martin-Smith, 1998). Conversely *Rasbora hubbsi* and *R. sumatrana* inhabit substantially different microhabitats (Martin-Smith, 1998), yet have similar seasonal patterns of GSI. The limited data available suggest that both congeneric species pairs feed on similar items (Inger and Chin, 1962; Martin-Smith, unpubl. data). One significant ecological difference between *G. danumensis* and *G. lepidogaster* is that the former is far more site-associated than the latter (Martin-Smith *et al.*, in press), although the implications of this finding for reproduction remain unclear. Both *G. danumensis* and *R. hubbsi*, despite being substantially smaller than their congeners (< 50 mm compared to > 90 mm SL), showed much higher investment in reproduction. This may indicate an irreducible minimum investment for successful reproduction which would lead to a higher GSI in very small species.

The two species showing the most extreme seasonality in GSI were *G. borneensis* and *L. bo*. These species are ecologically similar, inhabiting similar habitats and feeding on benthic algae and detritus (Inger and Chin, 1962; Martin-Smith, 1998). It is probable that benthic production decreases during the wet season due to increased turbidity, that egg

and fry predation reduces for the same reason and that the amount of spawning habitat increases. Higher production during the drier season may be necessary for initiation of gametogenesis. Finally *G. borneensis* and *L. bo* are the largest and presumably longest-lived species studied (along with *Osteochilus chini*) and thus will have further opportunities for reproduction if one season proves unsuccessful.

*Nematabramis everetti*, *Rasbora hubbsi* and *R. sumatrana* which all show year-round reproduction share a number of important ecological similarities. They are all surface feeding insectivores (Inger and Chin, 1962) and are small-medium sized species. Their food source may be more homogeneous distributed throughout the year. If their lifespan is only 6-18 months (Martin-Smith, unpubl. data) then seasonal reproduction may represent a far riskier strategy than continuous reproduction.

Winemiller (1989) examined life history traits for 71 species from the seasonal llanos in Venezuela and proposed three groups with particular suites of characteristics: (1) parental care and seasonal reproduction; (2) small size, early maturation, continuous reproduction with small clutches; and (3) synchronised reproduction during early wet season, high fecundity, no parental care, breeding migrations. The 10 species studied here could generally be placed in the second and third groups except that no breeding migrations are known to take place. The balitorids have been shown to be restricted in their movements (Martin-Smith *et al.*, in press) and any spawning movements are likely to be strictly local, which is hypothesised to favour multiple spawning (Lowe-McConnell, 1987). However, large, mature *L. bo* may move some distance from main rivers into tributaries during spawning periods (Martin-Smith, unpubl. data).

Lowe-McConnell (1987) states that females are generally larger than males where fecundity is at a premium. Six of the ten species investigated showed significantly larger females than males, although this did not correspond to whether the species was a presumed total or partial spawner. Given the stochastic nature of physical conditions high fecundity would be necessary to assure reproductive success.

In conclusion, three or four different reproductive patterns have been demonstrated for fishes from Sabah. Some species showed seasonal maturation of gonads with brief or extended periods of high GSI while other species showed year-round maturation of gonads. Reproductively mature individuals of these latter species were found throughout the year. Reproduction appeared to be correlated with the rainy season for a number of the seasonal species.

**Acknowledgements.** - We would like to acknowledge Yayasan Sabah (Forestry Upstream Division), Danum Valley Management Committee, State Internal Affairs & Research of Sabah Chief Minister's Department, Sabah Fisheries Department and Economic Planning Unit of the Prime Minister's Department for permission to conduct research in Sabah. Our collaborators, the Sabah Fisheries Department and Abdullah Samat of Universiti Kebangsaan Malaysia have been most helpful. The help of Dr. Adrian Marshall, the field assistance of Johnny Salleh bin Otong and Karim Kasil, the criticisms of two anonymous reviewers are gratefully acknowledged. Numerous volunteers from Raleigh International and Stephanie Hughes counted eggs. This work has been funded by ODA-NERC, The Royal Society, Small Project Grant 1228B from the British Ecological Society, and Grant No. 95/31/13 from the 100% Fund of Fauna and Flora International. This is part of Danum Valley Research and Training Programme project no. 105 and publication no. A/207 of the Royal Society Southeast Asian Rainforest Programme.

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Appendix I. - Sexual size-dimorphism for nine species of fish from Sabah. SL = upper limit of size class; % F = % females; % M = % males; n = number of individuals.

<i>G. borneensis</i>			<i>G. danumensis</i>			<i>G. lepidogaster</i>			<i>H. stephensoni</i>			<i>N. everetti</i>			<i>O. chini</i>			<i>P. sealei</i>			<i>R. hubbsi</i>			<i>R. sumatrana</i>		
SL	%F	%M	SL	%F	%M	SL	%F	%M	SL	%F	%M	SL	%F	%M	SL	%F	%M	SL	%F	%M	SL	%F	%M	SL	%F	%M
55	0	10	22	0	3	25	0	4	40	0	4	60	0	2	70	0	15	45	0	3	27	0	2	60	0	2
60	7	10	24	1	8	30	0	1	43	2	6	65	3	2	80	0	7	50	0	6	30	0	2	65	1	12
65	10	13	26	10	16	35	0	7	46	4	17	70	11	10	90	1	4	55	0	9	33	0	8	70	3	27
70	21	17	28	20	22	40	0	21	49	10	33	75	26	22	100	3	11	60	1	9	36	1	20	75	10	32
75	22	35	30	25	20	45	0	15	52	23	31	80	30	32	110	9	22	65	3	9	39	5	30	80	28	19
80	20	12	32	22	21	50	3	17	55	29	4	85	20	16	120	6	22	70	10	3	42	8	26	85	33	8
85	13	5	34	15	7	55	13	13	58	19	2	90	8	10	130	20	7	75	15	18	45	27	12	90	17	0
90	3	0	36	2	3	60	38	18	61	7	2	95	2	5	140	24	11	80	22	26	48	38	0	95	6	0
95	2	0	38	6	0	65	36	4	64	4	0	100	0	1	150	20	0	85	11	12	51	14	0	100	1	0
100	0	0				70	10	0	67	1	0				160	11	0	90	17	0	54	6	0			
105	0	0				75	0	0	70	1	0				170	4	0	95	13	6						
110	0	0				80	1	0							180	1	0	100	7	0						
																		110	1	0						
n	249	84		106	112		96	96		124	78		391	291		70	54		92	74		84	50		193	90

## AGE AND GROWTH OF THE STARSPOTTED SMOOTH-HOUND, *MUSTELUS MANAZO* (CHONDRICHTHYES: TRIAKIDAE) IN THE WATERS OF NORTH-EASTERN TAIWAN

by

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**ABSTRACT.** - Age and growth of the starspotted smooth-hound, *Mustelus manazo*, were examined from centrum analysis using soft x-radiography. Based upon seasonal centrum edge analysis, dark (opaque) ring was formed annually, mostly in July. Data were fitted to von Bertalanffy growth equations; mean lengths at each age were back-calculated with nonlinear regression technique. The growth until age 4 was similar in both sexes, but over this age, females tended to grow larger than males. The observed maximum lengths was 707 mm TL for males and 865 mm TL for females, and the maximum ages were age 5 for males and age 9 for females. The specimens attained their first maturity at 547 mm TL (age 2) for males and 597 mm TL (age 2) for females. All specimens over 640 mm TL (age 4) for males and over 700 mm TL (age 5) for females were fully mature.

**RÉSUMÉ.** - Âge et croissance de l'émissolle étoilée, *Mustelus manazo* (Chondrichthyes: Triakidae), dans les eaux nord-orientales de Taiwan.

L'âge et la croissance de l'émissolle étoilée, *Mustelus manazo*, ont été déterminés en examinant les corps vertébraux par radiographie. L'analyse saisonnière du bord des corps vertébraux a montré que les anneaux sombres (opaques) étaient formés annuellement, principalement en juillet. L'équation de von Bertalanffy s'appliquait parfaitement aux tailles calculées par régression non-linéaire. Jusqu'à 4 ans, la croissance est identique pour les individus des deux sexes, ensuite les femelles tendent à être plus grandes que les mâles. Les tailles maximales observées étaient de 707 mm LT pour les mâles et de 865 mm LT pour les femelles. Les mâles les plus âgés avaient 5 ans et les femelles 9 ans. Les mâles atteignent leur maturité pour une taille de 547 mm LT (âge 2) et les femelles pour une taille de 597 mm LT. Tous les mâles de taille supérieure à 640 mm LT (âge 4) et toutes les femelles supérieures à 700 mm LT étaient matures.

**Key-words.** - Triakidae, *Mustelus manazo*, Taiwan, Age, Growth, Maturity.

Two species of *Mustelus* occur in the Taiwanese waters, the starspotted smooth-hound, *M. manazo* Bleeker, 1854, and the spotless smooth-hound *M. griseus* Pietschmann, 1908. As shown by their common names, these two sharks are distin-

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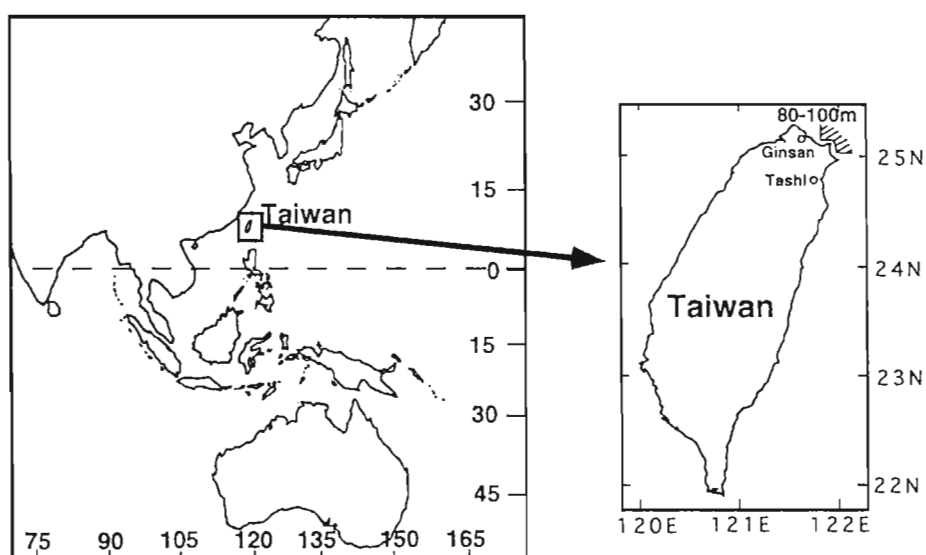


Fig. 1. - Sampling location off northeastern Taiwan. Oblique bars denote collecting area for the star-spotted smooth-hound, *Mustelus manazo*, by bottom longline and trawl in December 1994 - June 1996.

guished by their color pattern; *M. manazo* has numerous white spots on its flanks, whilst *M. griseus* is plain grey.

In the Western North Pacific, *M. manazo* is found from southern Siberia to Viet Nam (Compagno, 1984) and is one of the most abundant small coastal sharks in this area. In Taiwan and in Japan, it is commercially fished, mainly by bottom trawlers and longliners throughout the year. However, there is no fishery data available for this smooth-hound, neither in Taiwan, nor in Japan. According to Taiwanese fishermen, the catches have been decreasing during the last 15 years, so that this smooth-hound is nowadays exploited only in the waters off northeastern Taiwan.

Some aspects of the biology of *M. manazo* have been studied in Japanese waters by Tanaka and Mizue (1979) and Teshima (1981) in the East China Sea, by Taniuchi *et al.* (1983) off Choshi, and by Yamaguchi *et al.* (1996, 1997) in the Tokyo Bay.

The present study was carried out on the population of *M. manazo* from northeastern Taiwan in order to determine its age composition and growth.

## MATERIALS AND METHODS

Figure 1 shows the sampling location in the waters off northeastern Taiwan. The range of depths of this area is about 80-100 m. A total of 201 specimens (78 males and 123 females) were sampled from the catches of commercial bottom longliners and bottom trawlers between December 1994 and June 1996.

The largest centra, i.e., the 30th to 37th on the vertebral column, were sampled for age determination following Yamaguchi *et al.* (1996). The connective tissues were removed and the cleaned centra were preserved in 70% ethanol for several days. Then, they were sectioned, along the longitudinal axis, in half or in slices of about 1-2 mm thick. The slices were ground with a revolving wetstone.



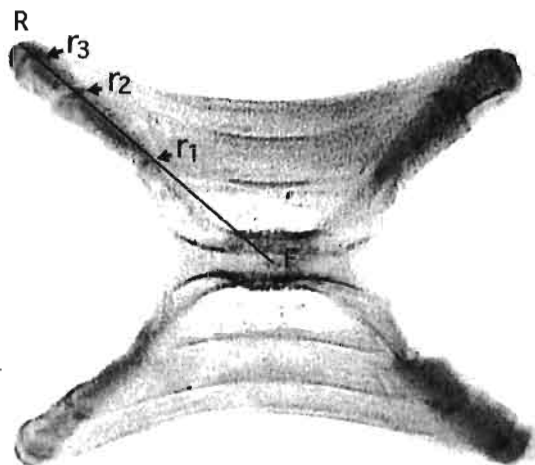


Fig. 2. - Soft X-radiograph of centrum in *Mustelus manazo*. R: Centrum radius; r1-3: Annual ring radii; F: Focus.

Soft x-radiographs (Fig. 2) were taken following Yamaguchi *et al.* (1996). The radius of each centrum was measured from the focus to the distal margin. The distances from the focus to each ring were measured at points along the internal margin of the corpus calcareum. The rings were counted two or more times, and only the consistent counts were retained.

The period of annual ring formation was determined by monthly changes in percentage occurrence of the centra with dark rings. The mean radii of each ring at each estimated age were calculated to determine the size-at-age by back-calculation. The von Bertalanffy growth equations were fitted to the mean back-calculated total length and standard errors at each age using computer program of Akamine (1986) which implements Marquardt's algorithm for nonlinear least squares parameter estimation.

Maturity stages were defined by Yamaguchi *et al.* (1997); immature males: claspers not calcified and testis not developed; premature males: claspers more or less calcified, and testis developed but no sperm in the seminal vesicle; mature males: claspers calcified and sperm in the seminal vesicle; immature females: uteri threadlike and no obviously developed ova in the ovary; premature female: uteri thickening but no obvious vascularization, developed ova more or less distinct; mature females: embryos present or uteri fully flaccid.

Clasper length was measured from the cloaca to the clasper tip.

## RESULTS AND DISCUSSION

High percentage of the centra were readable, i.e., 186 out of the 201 examined (93%). Figure 3 shows the period of ring formation determined by monthly changes in the percentage occurrence of dark (opaque) rings appearing on the edge of the centra; the frequency of dark rings have one large peak in July. So, these rings are annual and are mainly formed in July, i.e., somewhat later than the period of ring formation for *M. griseus* in Taiwanese waters, which takes place in April-June (Wang and Chen, 1982). The

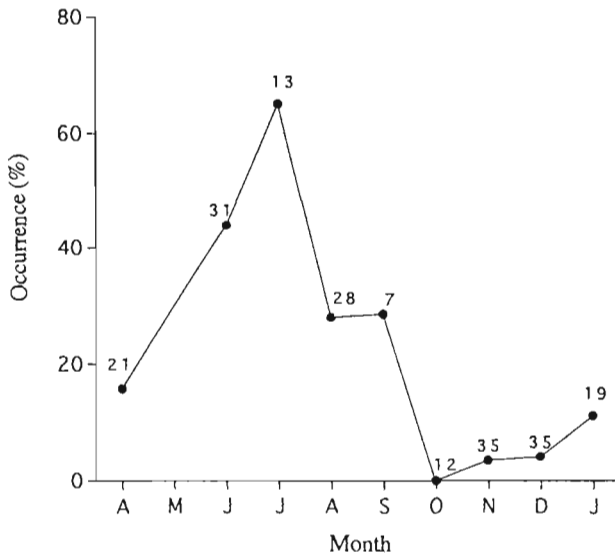


Fig. 3. - Monthly changes in percentage occurrence of centrum with dark ring appearing on the edge of the centra for the Taiwanese population of *Mustelus manazo*. The numbers in the figure denote the number of readable specimens.

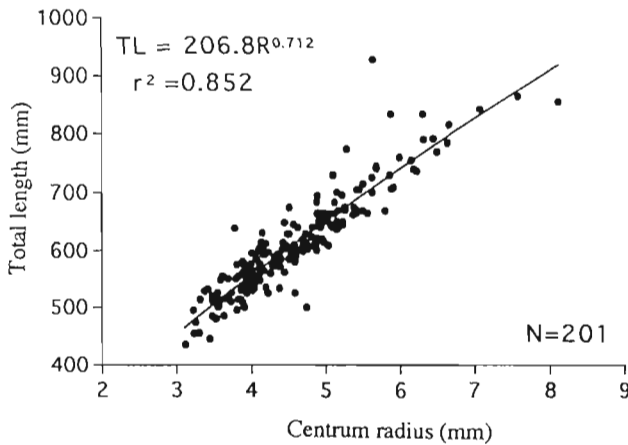


Fig. 4. - Relationship between the centrum radius size and the total length of *Mustelus manazo* from Taiwan.

period of ring formation for *M. manazo* in Taiwanese waters is similar to that determined for the Japanese population of *M. manazo* (Tanaka and Mizue, 1979; Taniuchi *et al.*, 1983; Yamaguchi *et al.*, 1997). There is a significant correlation (Fig. 4) between the total length and the size of the centrum radius. An analysis of covariance showed no significant difference between sexes, therefore these data were combined.

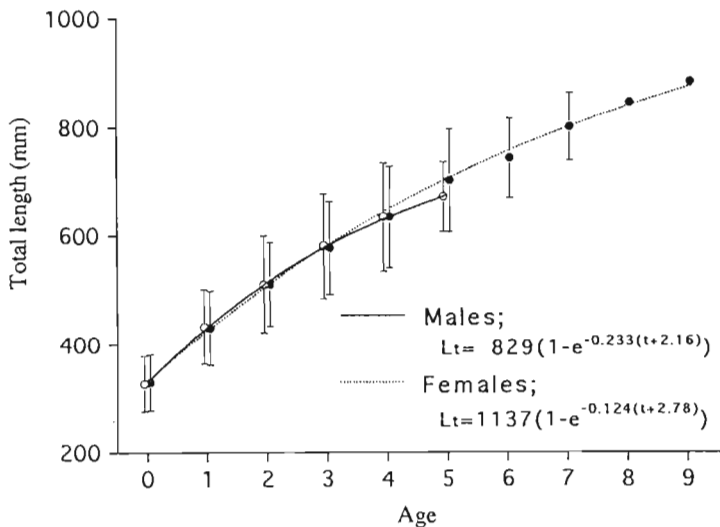


Fig. 5. - The von Bertalanffy growth curves for males and females of *Mustelus manazo* from Taiwan. The circles and the bars denote mean back-calculated length at each age and 95% confidence intervals of back-calculated length for males and females, respectively.

No new born specimens were found during this study, however full-term embryos were observed in April (mean total length 240 mm, range 225-278 mm,  $n = 11$ ). No embryo was found in June. Thus, embryos should be hatched in May-June, at a size of 250-300 mm TL.

Back calculation from ring formation data allowed to determine the size at the first ring formation to about 327 mm TL for the males and 329 mm TL for the females. This means that the first ring is formed soon after birth, and that the specimens with  $n$  rings are  $n-1$  year old. In Japanese waters, East China Sea and Tokyo Bay, the first ring is formed soon after birth (Tanaka and Mizue, 1979; Yamaguchi *et al.*, 1996).

Table I shows the size data of the ring radii for each age group, which were used to determine the von Bertalanffy equations (Fig. 5) for males and females of *M. manazo* in Taiwanese waters:

$$\begin{aligned} \text{females: } Lt &= 1137(1 - e^{-0.124(t+2.78)}) \\ \text{males: } Lt &= 829(1 - e^{-0.233(t+2.16)}) \end{aligned}$$

with:  $t$  = age in years;  $Lt$  = total length in mm at age  $t$ .

In both sexes, the growth was similar until age 4, but over this age, females tended to grow larger than males. These trends have been reported for *M. manazo* from Japan (Taniuchi *et al.*, 1983; Yamaguchi *et al.*, 1996) and many *Mustelus* species (Francis, 1981; Massey and Francis, 1989; Lenanton *et al.*, 1990; Yudin and Cailliet, 1990; Francis and Francis, 1992; Moulton *et al.*, 1992).

The oldest male in the specimens examined was age 5, and the oldest female was age 9. The greatest male had 707 mm TL and the greatest female 865 mm TL. It is assumed that the life span of the Taiwanese population is relatively short since maximum ages were the lowest.

Taiwanese specimens of both sexes are generally smaller than specimens of Japanese populations (Tanaka and Mizue, 1979; Taniuchi *et al.*, 1983; Yamaguchi *et al.*, 1997).

Table I. - Mean centrum ring radii and standard errors for each age group of *Mustelus manazo* from Tawain.

Males		Mean ring radii $\pm$ SE (mm)									
Age groups	N	r1	r2	r3	r4	r5	r6	r7	r8	r9	r10
1	4	1.82 $\pm$ 0.15	2.89 $\pm$ 0.22								
2	25	1.86 $\pm$ 0.15	2.79 $\pm$ 0.19	3.51 $\pm$ 0.30							
3	20	1.91 $\pm$ 0.11	2.84 $\pm$ 0.26	3.61 $\pm$ 0.31	4.27 $\pm$ 0.32						
4	18	1.94 $\pm$ 0.15	2.84 $\pm$ 0.18	3.63 $\pm$ 0.29	4.33 $\pm$ 0.37	4.89 $\pm$ 0.37					
5	4	2.08 $\pm$ 0.05	2.85 $\pm$ 0.14	3.63 $\pm$ 0.13	4.13 $\pm$ 0.09	4.68 $\pm$ 0.16	5.18 $\pm$ 0.19				
Weighted mean	71	1.90 $\pm$ 0.14	2.83 $\pm$ 0.21	3.58 $\pm$ 0.30	4.28 $\pm$ 0.33	4.84 $\pm$ 0.35	5.18 $\pm$ 0.19				
Back-calculated length (mm)		327	433	511	581	634	671				
Females		Mean ring radii $\pm$ SE (mm)									
Age groups	N	r1	r2	r3	r4	r5	r6	r7	r8	r9	r10
1	3	1.79 $\pm$ 0.16	2.76 $\pm$ 0.07								
2	42	1.88 $\pm$ 0.11	2.82 $\pm$ 0.20	3.56 $\pm$ 0.26							
3	23	1.93 $\pm$ 0.10	2.76 $\pm$ 0.22	3.60 $\pm$ 0.26	4.24 $\pm$ 0.29						
4	31	1.97 $\pm$ 0.16	2.79 $\pm$ 0.16	3.50 $\pm$ 0.18	4.19 $\pm$ 0.24	4.75 $\pm$ 0.29					
5	6	2.06 $\pm$ 0.17	2.92 $\pm$ 0.35	3.68 $\pm$ 0.31	4.44 $\pm$ 0.40	5.15 $\pm$ 0.43	5.69 $\pm$ 0.51				
6	6	1.93 $\pm$ 0.08	2.85 $\pm$ 0.18	3.60 $\pm$ 0.10	4.19 $\pm$ 0.12	4.76 $\pm$ 0.17	5.41 $\pm$ 0.14	5.91 $\pm$ 0.19			
7	2	1.93	2.99	3.86	4.44	5.05	5.66	6.02	6.53		
8	1	1.85	2.57	3.49	3.85	4.99	5.68	6.44	6.95	7.19	
9	1	1.93	2.96	3.58	4.32	5.02	5.68	6.23	6.73	7.14	7.24
Weighted mean	115	1.92 $\pm$ 0.14	2.81 $\pm$ 0.21	3.56 $\pm$ 0.24	4.23 $\pm$ 0.28	4.83 $\pm$ 0.32	5.57 $\pm$ 0.33	6.02 $\pm$ 0.23	6.69 $\pm$ 0.18	7.17	7.24
Back-calculated length (mm)		329	431	511	577	634	701	742	800	845	883

For both sexes, the growth coefficient  $K$  estimated for the Taiwanese population of *M. manazo* was lower than that for the East China Sea population (Tanaka and Mizue, 1979), but higher than that for Choshi (Taniuchi *et al.*, 1983) and Tokyo Bay populations (Yamaguchi *et al.*, 1996) for both sexes. That means that the growth rate in Taiwan was relatively high. However, Cailliet *et al.* (1990) pointed out that the apparent differences in the growth rate between populations might not be real, and might be influenced by other factors, such as the sample sizes, preparation techniques and reading precision. Similarly, the study of the differences in growth between two populations of blue shark, *Prionace glauca*, might be procedurally and statistically invalid (Cailliet and Tanaka, 1990). The preparation techniques used for the study of the East China Sea population (Tanaka and Mizue, 1979) and that of Choshi (1983) were different from the soft x-radiography method used in the present study, therefore, the differences in growth rates should be carefully interpreted.

Table II. - Length and age at sexual maturity of *Mustelus manazo* in Taiwan.

Total length (mm)	Males			Females		
	Immature	Premature	Mature	Immature	Premature	Mature
421-440				1		
441-460	3					
461-480				2		
481-400		1		3	3	
501-520	7			7	1	
521-540	5	1		4	6	
541-560	2	3	2	6	7	
561-580	6		2	1	9	
581-600		2	6	2	5	4
601-620		1	11		8	2
621-640		2	1		5	4
641-660			8		3	3
661-680			10		1	7
681-700			2		1	2
701-720			2			4
721-740						6
741-760						4
761-780						2
781-800						3
801<						6
Total	23	10	44	26	49	47
	77			122		

Age	Males			Females		
	Immature	Premature	Mature	Immature	Premature	Mature
1	4			2	1	
2	15	7	3	20	19	3
3	2	3	15	2	14	7
4			18		14	17
5			4			6
6						6
7						2
8						1
9						1
Total	21	10	40	24	48	43
	71			115		

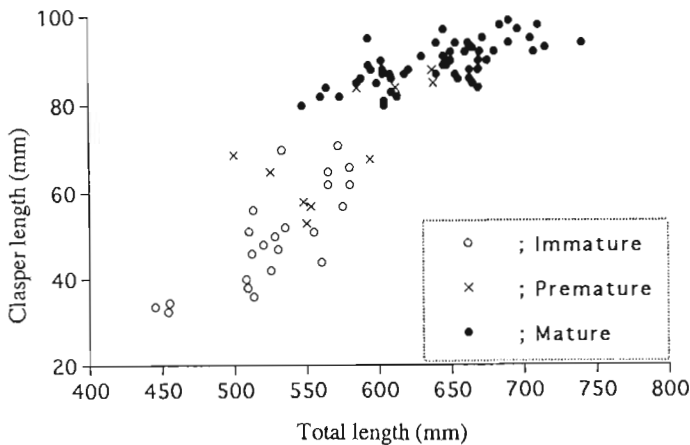


Fig. 6. - Relationship between total length and clasper length of *Mustelus manazo* in Taiwan.

Table II shows the length frequencies at 20 mm intervals at each maturity stage: males attained their first maturity at 547 mm TL and females at 597 mm TL. All males over 640 mm TL and all females over 700 mm TL were fully mature. Similarly, the specimens of both sexes attained their first maturity at age 2. All males over age 4 and all females over age 5 were fully mature. The length at sexual maturity was similar to that estimated for *M. griseus* in Taiwan (Wang and Chen, 1981), and the observed maximum total length was also almost the same (Wang and Chen, 1982). The length at sexual maturity is smaller than that estimated for Japanese population of *M. manazo*, and the age at sexual maturity is the lowest (Taniuchi *et al.*, 1983; Yamaguchi *et al.*, 1997).

As for the other chondrichthyans, the claspers of *M. manazo* increase with the total length (Fig. 6). Immature males of the Taiwanese population had short and soft claspers, they lengthened slowly with respect to total length. The claspers of adolescent males elongated rapidly and the full calcification was observed for males of about 600 mm TL. Over 800 mm TL, the clasper length showed little elongation.

As a result, the Taiwanese population of *M. manazo* has faster growth, shorter life span, faster maturity and the specimens are smaller than those of the Japanese population.

**Acknowledgements.** - We are grateful to Dr. Shoon-Jeng Joung and his students of the Taiwan Ocean University for his help in examining the Taiwanese material. This study was supported, in part, by a Grants-in Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (09NP0901).

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### Symposium 13

## POPULATION AND COMMUNITY ECOLOGY

Chairperson: Yves LETOURNEUR

- KOCHZIUS M. - Interrelation of ichthyofauna from a seagrass meadow and coral reef in the Philippines. 517-535

*Relations entre l'ichtyofaune d'un herbier de phanérogames et celle d'un récif corallien aux Philippines.*

- SCHMITT R.J. & S.J. HOLBROOK. - Temporal patterns of settlement of three species of damselfish of the genus *Dascyllus* (Pomacentridae) in the coral reefs of French Polynesia. 537-551

*Chronologie de l'installation de trois espèces de poissons-demoiselles du genre Dascyllus (Pomacentridae) sur les récifs coralliens de Polynésie française.*

- WOODLAND D.J. - An examination of the effect of ecological factors, especially competitive exclusion, on the distributions of species of an in-shore, tropical, marine family of Indo-Pacific fishes (Siganidae). 553-562

*Analyse de l'influence des facteurs écologiques, notamment de l'exclusion par compétition, sur les distributions des espèces d'une famille de poissons tropicaux littoraux de l'Indo-Pacifique, les Siganidae.*



## INTERRELATION OF ICHTHYOFAUNA FROM A SEAGRASS MEADOW AND CORAL REEF IN THE PHILIPPINES

by

Marc KOCHZIUS (1)

**ABSTRACT.** - Tropical seagrass meadows associated with coral reefs are utilised both daily and seasonally as feeding ground and nursery area by reef fishes. The interrelation of seagrass meadow and coral reef ichthyofauna was studied in Malatapay, Negros Oriental, Philippines. Sampling was conducted with a beach seine in a seagrass meadow at two distances (50 m and 250 m) from a coral reef at day and night. 115 species belonging to 70 genera and 42 families were identified. Hierarchical clustering by Bray-Curtis similarity of samples shows a day and a night group with sub-groups depending on distance to the coral reef. Additional observations by visual censuses support these results. Resident and diurnally active species of the seagrass meadow belong to the families Labridae, Scaridae, Siganidae, Mullidae, Pomacentridae and Gobiidae. Nocturnally active and resident species belong to the families Bothidae, Soleidae, Callionymidae, Scorpaenidae and Lethrinidae. Apogonidae, Plotosidae, Holocentridae, Diodontidae, Congridae, Muraenidae, Ophichthidae and Tetraodontidae migrate into the seagrass meadow at night to forage. Length frequencies indicate that Mullidae, Scorpaenidae, Lethrinidae and Plotosidae utilise the seagrass meadow as a nursery area. This demonstrates the interrelation of these habitats and the importance of seagrass meadows for coral reef fishes.

**RÉSUMÉ.** - Relations entre l'ichtyofaune d'un herbier de phanérogames et celle d'un récif corallien aux Philippines.

Les herbiers de phanérogames tropicaux qui sont associés aux récifs coralliens sont utilisés par les poissons du récif comme aire d'alimentation et de nurserie. Cette étude examine les corrélations entre les herbiers de phanérogames et les poissons du récif corallien à Malatapay, Negros Oriental, Philippines. L'échantillonnage a été réalisé avec une senne de plage dans l'herbier, à 50 et 250 m du récif corallien. Les échantillons ont été pris pendant le jour et la nuit. 115 espèces appartenant à 70 genres et 42 familles ont été identifiées. La classification hiérarchique de similarité de Bray-Curtis des échantillons met en évidence un groupe d'espèces diurnes et un groupe d'espèces nocturnes avec des sous-groupes dépendant de la distance au récif corallien. Les observations visuelles confirment ce résultat. Les poissons ayant une activité diurne appartiennent aux familles suivantes: Labridae, Scaridae, Siganidae, Mullidae, Pomacentridae et Gobiidae, et les espèces ayant une activité nocturne font partie des Bothidae, Soleidae, Callionymidae, Scorpaenidae et Lethrinidae. Les Apogonidae, Plotosidae, Holocentridae, Diodontidae, Congridae, Muraenidae, Ophichthidae et Tetraodontidae migrent vers l'herbier durant la nuit pour y rechercher de la nourriture. Les fréquences de tailles indiquent que les Mullidae, Scorpaenidae, Lethrinidae et Plotosidae utilisent l'herbier comme aire de nurserie. Cela montre les rapports étroits entre ces habitats et l'importance des herbiers pour les poissons du récif.

**Key-words.** - Ichthyofauna, ISEW, Philippines, Seagrass meadow, Coral reef, Migration, Feeding ground, Nursery area.

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Tropical seagrass meadows are known as important nursery areas and feeding ground for fishes from adjacent coral reefs. They are seasonal residents in their early life stages or diurnal migrating visitors (Jones and Chase, 1975; Weinstein and Heck, 1979; Ogden and Gladfelter, 1983; Heck and Weinstein, 1989; Parrish, 1989). Many reef fishes migrate from reefs at night into seagrass meadows to forage, and are followed by piscivores. These predators from the reef are more common in seagrass meadows associated with coral reefs than in seagrass meadows associated with other habitats (Weinstein and Heck, 1979; Robblee and Zieman, 1984; Bell and Pollard, 1989). Due to these migrations the composition of seagrass meadow fish fauna is influenced by adjacent coral reefs. Connection of these habitats by fishes leads to a transport of biomass, organic substance and nutrients from seagrass meadows into coral reefs (Ogden, 1980; Ogden and Gladfelter, 1983; Meyer and Schulz, 1985; Parrish, 1989; Brouns and Heijs, 1991). Studies in the Caribbean have shown that the biomass of fishes is higher in coral reefs adjacent to seagrass meadows than in reefs without seagrass beds (Birkeland, 1985). Comparison of fisheries from different coral reef regions suggests that coral reefs with extended shallow water habitats, such as seagrass meadows or mangroves, yield the highest catch. Reefs with a ratio of shallow water habitat to coral reef of 1:1 or more are very productive (Marshall, 1985).

In the Philippine coastal waters seagrass meadows cover an area of 10,000 to 50,000 km<sup>2</sup>. Transfer of findings from the well investigated Caribbean seagrass meadows to the Philippines suggest that 85% of commercial important fish species spend a part of their life cycle in seagrass meadows (Thorhaug and Cruz, 1988).

This study was conducted to investigate spatial and temporal patterns of seagrass meadow fish fauna and its relationship to the fish community of adjacent coral reefs.

## MATERIAL AND METHODS

The study site Malatapay is located in the southern part of Negros Oriental (9°07'N, 123°12'E) adjacent to the Mindanao Sea in the Central Visayas Region, Philippines (Fig. 1). The seagrass meadow studied is situated between two coral reefs (Fig. 1) and is mainly composed of the seagrass species *Syringodium isoetifolium*, *Halodule univervis* and *Cymodocea serrulata*. Water depth ranged from 1.0 to 10.0 m, with an average depth of 3 m at the sampled stations. The sampled area includes the sand flat between the seagrass meadow and the beach. The reference coral reef in this study is located at the right side of the seagrass meadow, close to station 1 (Fig. 1). It is assumed that the artificial reef does not influence station 2, because it is in a poor condition. The constructions of tires do not show any overgrowth with corals or other benthic organisms and only a small fish community exists.

Sampling was conducted from July to September 1995 with a beach seine of 30 m length, 1.5 - 2.5 m height and with a mesh size of 2 mm. A total of 74 samples were taken in the seagrass meadow during six 24 h cycles in two different distances (50 m and 250 m) from the coral reef (Table I). The length of each specimen was recorded and, if preservation was necessary, samples were frozen. Species identification was based on literature by Gloerfelt-Tarp and Kailola (1984), Lieske and Myers (1994), Randall *et al.* (1990) and Rau and Rau (1980).

During day, additional data were collected with the visual census technique based on English *et al.* (1994). A total of 36 visual censi was conducted by snorkelling on a

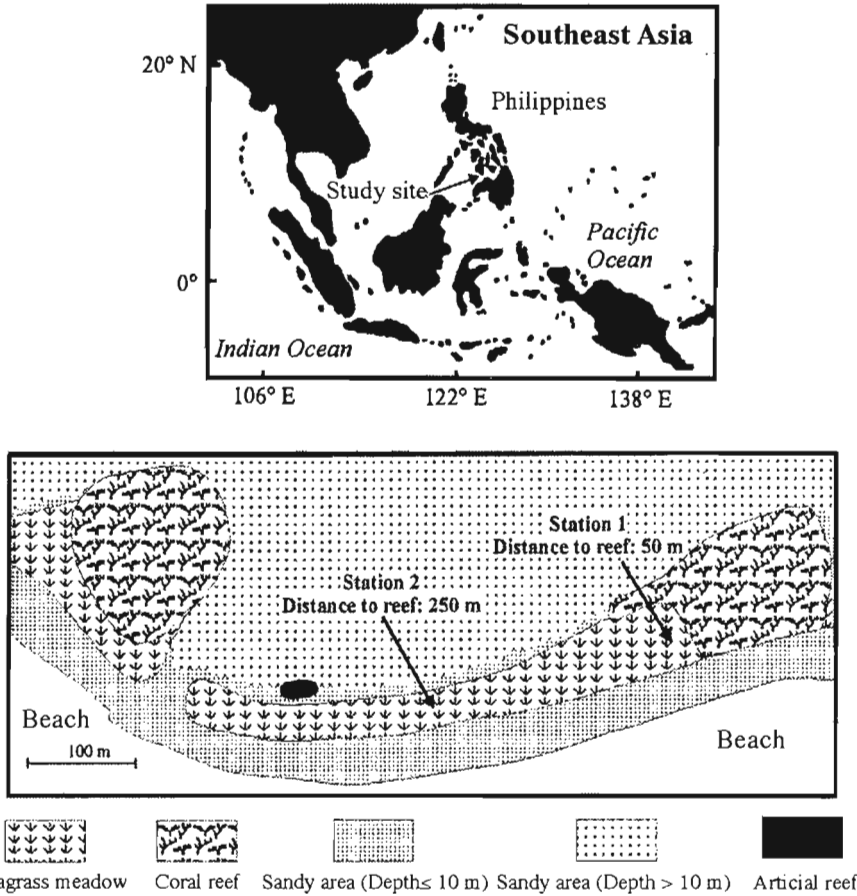


Fig. 1. - Study site. A: map of Southeast Asia; B: Bay of Malatapay, Negros Oriental, Philippines.

Table 1. - Sampling strategy: number of samples taken in the seagrass meadow by beach seine and visual census at station 1 and station 2 during day and night (sunset: 18:30; sunrise: 5:30).

	Day			Night		
	After sunrise	Noon	Before sunset	After sunset	Mid-night	Before sunrise
	7:00-7:30	12:00-12:30	17:30-18:00	20:00-20:30	00:00-00:30	4:30-5:00
Beach seine, station 1, 50 m distance to coral reef	6	6	6	6	6	6
Beach seine, station 2, 250 m distance to coral reef	6	6	6	6	6	6
Visual census, station 1, 50 m distance to coral reef	6	6	6	—	—	—
Visual census, station 2, 250 m distance to coral reef	6	6	6	—	—	—

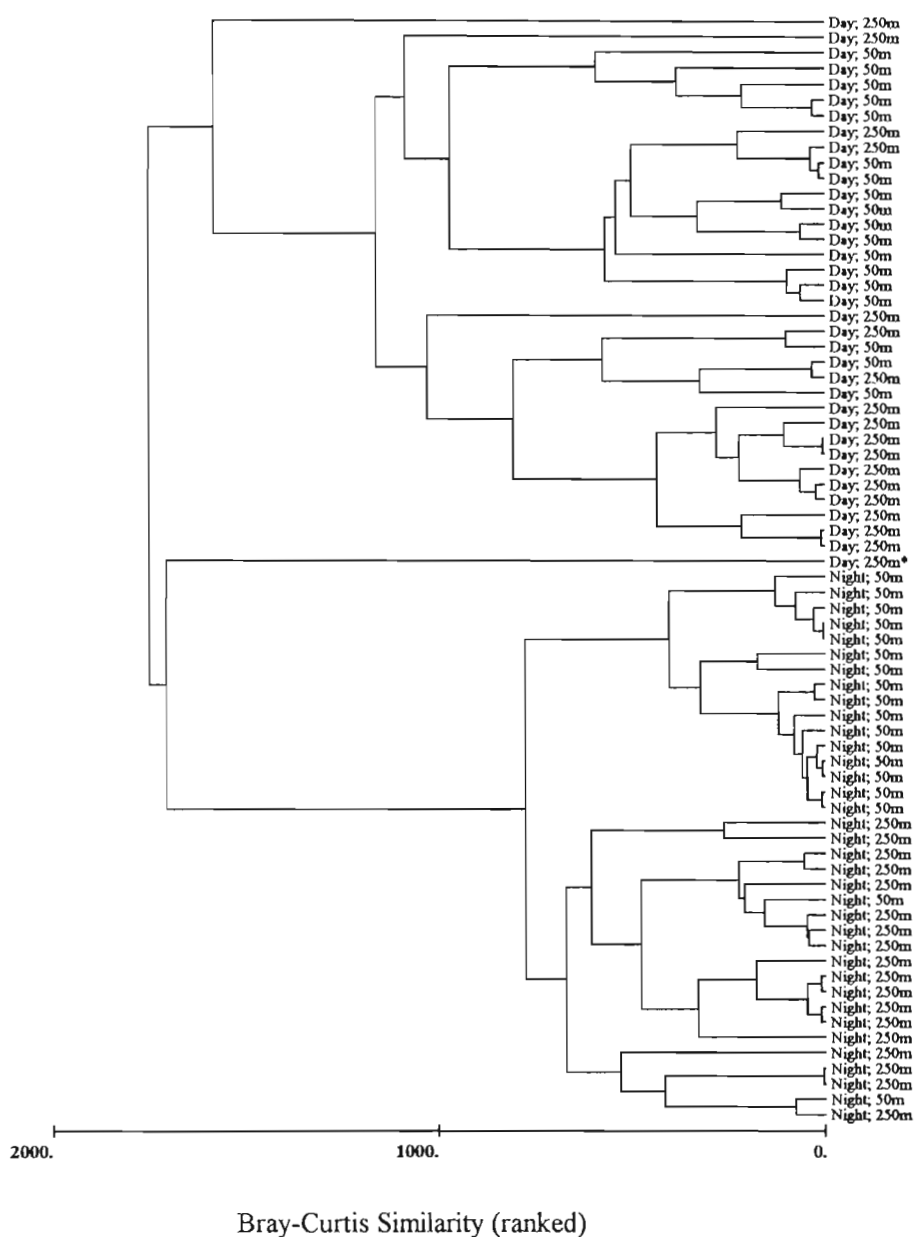


Fig. 2. - Hierarchical clustering: Sample similarity dendrogram of beach seine samples ( $n = 72$ ;  $\sqrt[3]{x}$  transformation of data). Day; 50 m = day / distance to coral reef: 50 m. Day; 250 m = day / distance to coral reef: 250 m. Night; 50 m = night / distance to coral reef: 50 m. Night; 250 m = night / distance to coral reef: 250 m.

100 m transect with a width of 10 m ( $= 1000 \text{ m}^2$ ) at the same stations where the beach seine was used (Table I). Four additional visual censi (50 x 5 m transects) were conducted

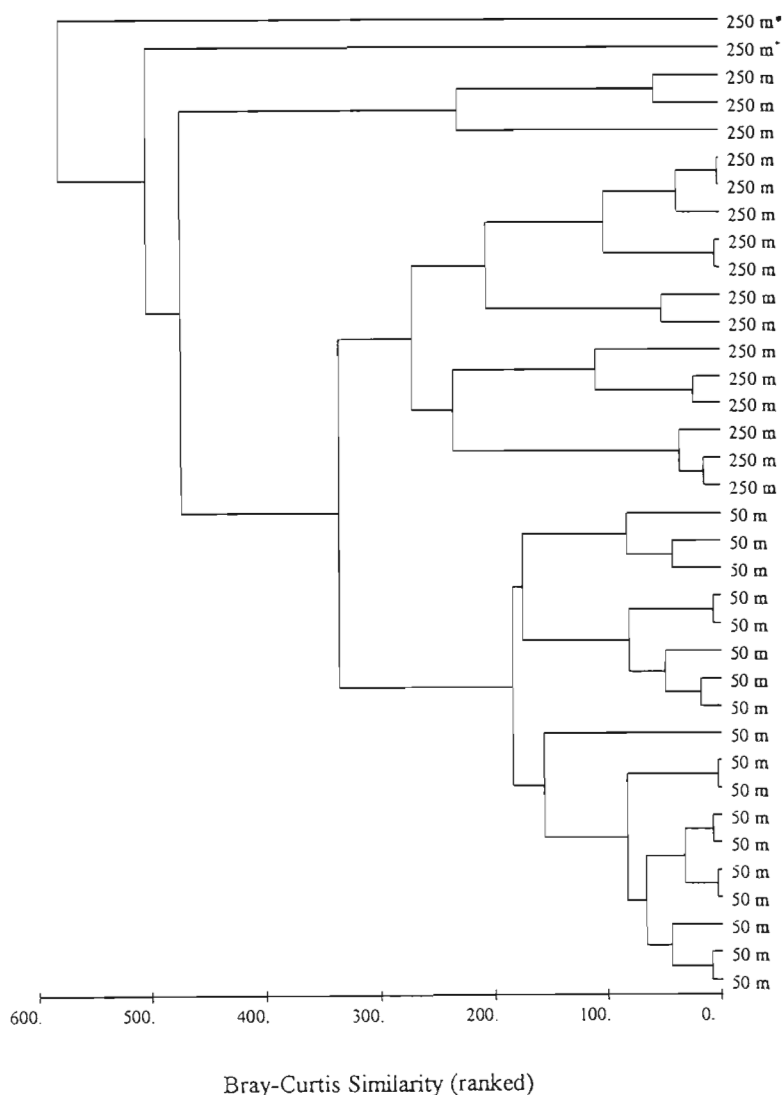


Fig. 3. - Hierarchical clustering: Sample similarity dendrogram of visual census data ( $n = 36$ ;  $\sqrt{\sqrt{}}$  transformation of data). 50 m = distance to coral reef: 50 m. 250 m = distance to coral reef: 250 m.

by B. Schirm and E. Cruz (CEMRINO) in both adjacent coral reefs at depth of 4 and 10 m. These data were used to compare size distribution of fishes between seagrass meadow and coral reef.

Hierarchical clustering and ANOSIM (Analysis of similarities) significance test are performed with PRIMER 4.0 software (Carr *et al.*, 1994). Hierarchical clustering of samples is based on Bray-Curtis similarities of  $\sqrt{\sqrt{}}$  (square root) transformed abundance data of species. Hierarchical clustering of species is based on Bray-Curtis similarities of  $\sqrt{\sqrt{}}$  transformed and standardised abundance data (Clarke and Warwick, 1994). The ANOSIM significance test compares the similarities of species composition between the samples

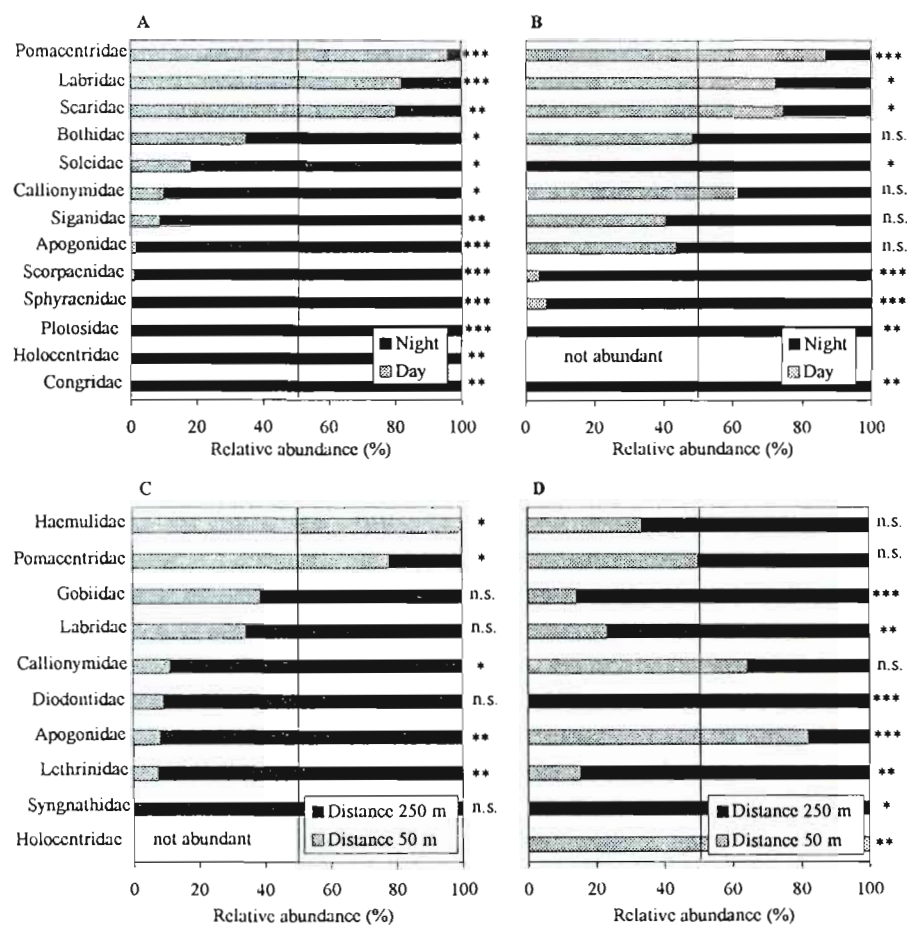


Fig. 4. - Diel (A, B) and spatial (C, D) variation in relative abundance of the seagrass meadow fish fauna in beach seine samples. **A:** Distance to coral reef 50 m; **B:** Distance to coral reef 250 m; **C:** Day; **D:** Night. Significance tested with Kruskal-Wallis ANOVA by ranks and median test: \* =  $0.05 \geq p \geq 0.01$ ; \*\* =  $0.01 > p \geq 0.001$ ; \*\*\* =  $p < 0.001$ ; n.s. = not significant.

and can give evidence for differences. Statistical significance tests such as Kruskal-Wallis ANOVA by ranks and median test were performed with STATISTICA software (Anon, 1993).

## RESULTS

Pooled data of beach seining and visual census recorded a total of 115 species belonging to 70 genera and 42 families (Appendix). Apogonidae, Labridae, Plotosidae and Scorpaenidae are the most abundant families in pooled beach seine samples. Pooled diurnal visual census samples show the following ranking in abundance: Pomacentridae, Siganidae, Labridae and Apogonidae.



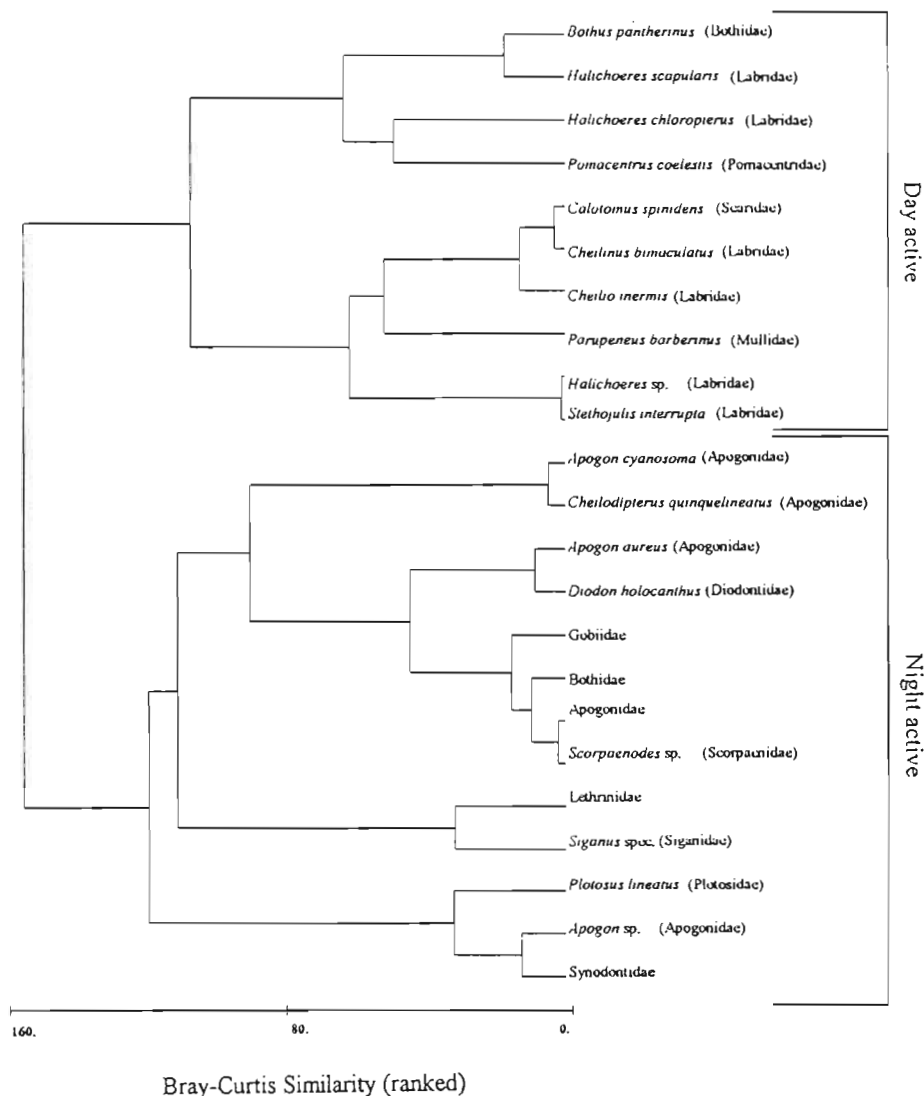


Fig. 5. - Species similarity dendrogram of beach seine samples ( $n = 72$ ; species or families  $> 10\%$  relative abundance in one sample).

Cluster analysis separates the beach seine samples into four clusters. Day and night cluster are divided into sub-cluster depending on distance to the coral reef (Fig. 2). Only one diurnal sample (Day; 250 m\*) differs from this grouping and is assigned to the nocturnal samples. This is caused by a school of juvenile Siganidae which occurred only in this sample. Normally Siganidae were abundant only in nocturnal beach seine samples. No statement can be given to mismatches according to distances within the day and night cluster. The difference between the distances to the coral reef within these clusters is con

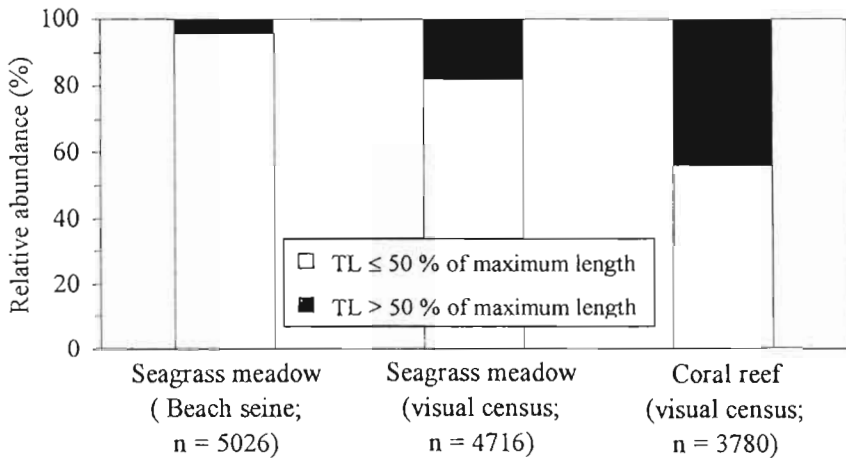


Fig. 6. - Relative abundance of juvenile fishes (TL  $\leq$  50% of maximum length) in the seagrass meadow and adjacent coral reef (pooled diurnal samples); maximum length based on Lieske and Myers (1994).

firmed with an ANOSIM significance test ( $p < 0.005$ ). No difference is detected between the different times of the day and different times of the night by the cluster analysis.

Cluster analysis of visual census data shows a separation in two clusters according to the distance to the coral reef (Fig. 3). Five samples of the distance category 250 m do not fit into this pattern. Sample 250 m\* shows the slightest similarity to all other samples because a school of 500 juvenile *Plotosus lineatus* was recorded. A reason for the poor similarity of sample 250 m+ was the low abundance of fishes in this census. The difference between the two distance categories is confirmed with an ANOSIM significance test ( $p < 0.005$ ).

Relative abundance of fish families in beach seine samples during day and night reflects different pattern of activity (Fig. 4). Pomacentridae, Labridae and Scaridae show a significant higher relative abundance during day at both stations. Congridae, Plotosidae, Holocentridae, Scorpaenidae, Apogonidae, Sphyraenidae, Callionymidae, Siganidae, Bothidae and Soleidae show a significant higher relative abundance at night at both or one of the different stations. Muraenidae, Ophichthidae and Tetraodontidae were observed in low numbers in the seagrass meadow at night (Appendix). A species similarity dendrogram of beach seine catches separates the most abundant species into a day and a night active group (Fig. 5). Species of the day active group belong to the families Labridae, Scaridae, Pomacentridae and Mullidae. Night active species are members of the families Apogonidae, Diodontidae, Bothidae, Scorpaenidae, Lethrinidae, Plotosidae, and Synodontidae.

The occurrence of Siganidae in nocturnal beach seine samples did not reflect a night active behaviour. Visual census data indicate a diurnal activity. Nocturnal observations show that siganid species hide and rest between the blades of the seagrass. This leads to an increased vulnerability to the beach seine at night. The classification of Gobiidae into the night active group is also caused by the increased efficiency of the net at night. Visual census data also indicate that Gobiidae belong to the day active group.

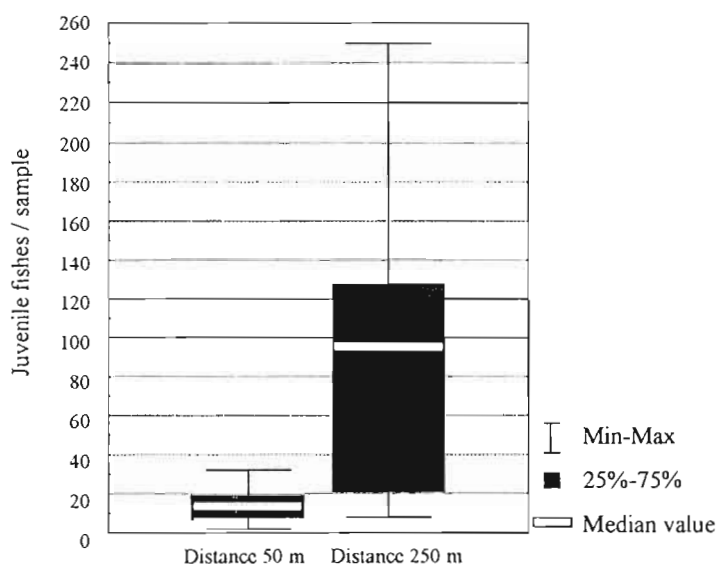


Fig. 7. - Number of juvenile fishes ( $\leq 50\%$  TL max.; Lieske and Myers, 1994) in diurnal beach seine samples (Box & Whisker Plot). Significant difference tested with Kruskal-Wallis ANOVA by ranks and median test ( $p < 0.001$ ).

Relative abundance of fishes in beach seine samples at the two stations reflects different spatial pattern (Fig. 4). Haemulidae and Pomacentridae are more abundant close to the coral reef at day. Callionymidae, Apogonidae and Lethrinidae show a higher relative diurnal abundance in a distance of 250 m from the corals. Nocturnal beach seine samples indicate that Gobiidae, Labridae, Diodontidae, Lethrinidae and Syngnathidae are more abundant in a distance of 250 m to the coral reef (Fig. 4). In contrast Apogonidae and Holocentridae occur in a higher relative abundance close to the reef at night.

Length frequencies of pooled diurnal samples indicate that 95.6% of fishes in beach seine catches and 82.0% of specimens in visual census samples are small and reach only 50% or less of their maximum size (based on Lieske and Myers, 1994) (Fig. 6). Conversely the proportion of these small, i.e., juvenile, individuals is only 56% (visual census) in the adjacent coral reef (Fig. 6). Juvenile fishes in beach seine samples show a median abundance of 95 individuals per catch in a distance of 250 m from the reef and a median abundance of 13.5 individuals per catch in a distance of 50 m during day (Fig. 7). This pattern of higher abundance far from the reef is confirmed by a Kruskal-Wallis ANOVA and median test with a significance level of  $p < 0.001$ . Length frequencies of Mullidae, Scorpaenidae, Lethrinidae and Plotosidae suggest that these families use the seagrass bed as a nursery area (Fig. 8). Beach seine samples of Mullidae show that 80% of all individuals belong to the size classes of 4, 5 and 6 cm (Fig. 8). More than 95% of Scorpaenidae and Lethrinidae in beach seine samples have a size of 2, 3 or 4 cm (Fig. 8). Adult individuals of Scorpaenidae and Lethrinidae are not or seldom observed in beach seine catches or by the visual census technique. 74% of Plotosidae belong to the size classes from 3 to 7 cm (Fig. 8). Juveniles of *Plotosus lineatus* occur in large schools of individuals of similar size. Adult individuals of *P. lineatus* are only observed as nocturnal visitors from the coral reef. Beach seine catches and visual census data show that juveniles of *P. lineatus* inhabit the seagrass meadow at day and night.

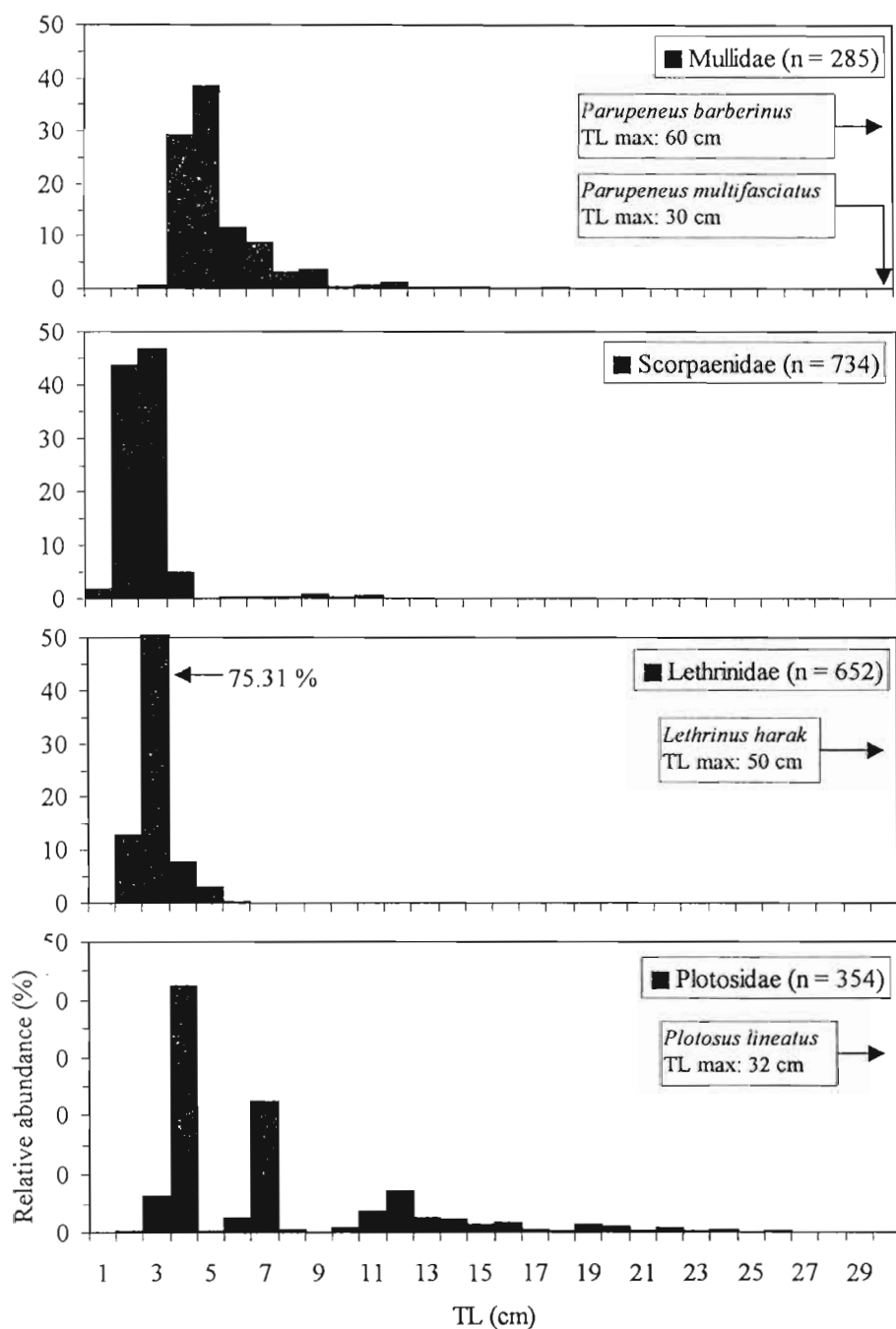


Fig. 8. - Length frequencies of Mullidae, Scorpaenidae, Lethrinidae and Plotosidae (Beach seine). Maximum length is given for the most abundant species (Lieske and Myers, 1994).

## DISCUSSION

In comparison to the ichthyofauna of seagrass beds in other tropical Indo-Pacific regions, the seagrass meadow fish fauna in Malatapay is well diversified: 115 species and 42 families (Jones and Chase, 1975; Hutomo and Martosewojo, 1977; Martosewojo, 1989; Dolar, 1991; Vegara and Fortes, 1991; Sudara *et al.*, 1992). A higher number of 186 species and 48 families was observed in nocturnal beam trawl catches in a seagrass bed close to a coral reef in Bolinao, Philippines (McManus *et al.*, 1992). Fortes (1990) recorded 123 species from 51 families in Philippine seagrass meadows. On the one hand the high number of species in this study in comparison to other studies is a result of the sampling methods. Samples were taken by beach seine and visual census during the day and at night. Other studies only conducted sampling with one method during day. On the other hand the adjacent coral reef leads to an increase of species by migrating coral reef fishes. This indicates that seagrass meadows in the vicinity of coral reefs inhabit more fish species than seagrass beds without adjacent reefs. The investigations of this study show that Apogonidae, Labridae and Siganidae are the most abundant families of the seagrass meadow fish fauna. Fortes (1990) reports similar findings, but he gave more importance to Gobiidae than to Labridae. In this study, Gobiidae are less abundant.

The day active fish community is mainly composed of resident species. These species belong to the families of Labridae, Scaridae, Siganidae, Pomacentridae and Gobiidae. The night active fish community is composed of resident species, that belong to families like Bothidae, Soleidae and Callionymidae. Nocturnally migrating families such as Apogonidae, Plotosidae, Holocentridae, Diodontidae, Congridae, Muraenidae, Ophichthidae and Tetraodontidae have a high influence on the composition of the seagrass meadow fish fauna close to the reef at night. This pattern is reflected by the fact that there is no mismatch in the cluster of the nocturnal beach seine catches in a distance of 50 m to the coral reef (Fig. 2). Especially Apogonidae migrate into the seagrass meadow after sunset. This is indicated by the high relative abundance of Apogonidae close to the reef at night (Fig. 4). These nocturnal visitors migrate into the seagrass bed to forage and can feed on the abundant crustacean fauna (Weinstein and Heck, 1979; Bell and Pollard, 1989). Nocturnal feeding migrations of fishes from coral reef into adjacent seagrass beds are also described by Ogden (1980), Robblee and Zieman (1984) and Quinn and Ogden (1984). Predators such as Muraenidae, Congridae, Ophichthidae and Tetraodontidae can migrate over large distances from the reef into the seagrass bed to forage. Migrating fishes are an important vector for the export of organic matter and nutrients from seagrass beds into adjacent coral reefs (Ogden, 1980; Ogden and Gladfelter, 1983; Meyer and Schulz, 1985; Parrish, 1989; Brouns and Heijs, 1991). This foraging behaviour underlines the importance of seagrass meadows as a feeding ground for coral reef fishes.

Different fish assemblages according to the distance from the coral reef during day were evidenced. This pattern can be caused by predatory pressure of piscivore fishes from the coral reef. Large predators from the coral reef might control the abundance of smaller predators in the seagrass meadow. Therefore, the abundance of resident piscivore fishes in the seagrass meadow is limited and the predatory pressure on other resident fishes and juveniles might be reduced (Ogden, 1980; Parrish, 1989). Juvenile specimens are less abundant close to the reef than farther away from the reef. The juvenile individuals stay far away from the reef most likely to avoid predation of piscivore fishes from the coral reef. A similar pattern was observed for juvenile Acanthuridae in Caribbean seagrass meadows (Sweatman and Robertson, 1994).

Length frequencies of the fish fauna imply that mainly juvenile specimens inhabit the seagrass bed, as shown by Jones and Chase (1975), Weinstein and Heck (1979), Ogden and Gladfelter (1983) and Parrish (1989). Mullidae, Scorpaenidae, Lethrinidae and Plotosidae use the seagrass meadow as a nursery area. Fish larvae can settle down in seagrass meadows and find food and protection against predators until they are mature. As juveniles or sub adults they migrate to the coral reef. In this situation, seagrass meadows can have a important function as a "waiting room" for settlers of the coral reef fish fauna (Parrish, 1989). This supports the importance of seagrass meadows as a nursery area for coral reef fishes. For this reason seagrass beds should be more valued as a important habitat for coral reef fishes. Conservation of coral reefs is an important task in many regions of the world, but less attention is given to seagrass meadows. It is proposed that marine reserves have a function as a source of recruits for the re-colonisation of adjacent coral reefs (Man *et al.*, 1995), but beside coral reefs, seagrass meadows also can provide recruits. Therefore, reserves for the protection of coral reefs should also include the adjacent biotops, such as seagrass meadows.

**Acknowledgements.** - This work was conducted in the framework of the project "Establishment of Marine Reserves in Negros Oriental" funded by the European Union (DG1 Project B7-5040/94/09/1). The "Stifterverband für die Deutsche Wissenschaft" gave me support to take part in the 5th International Indo-Pacific Fish Conference. I would like to thank my colleagues from "Centre for the Establishment of Marine Reserves in Negros Oriental" (especially B. Schirm and M. Waltemath) and ZMT (especially Dr. Ekau and Prof. Saint-Paul) for their support. I am also grateful to Ulrike Schäfer for translation of the "résumé" and to the fisherfolks of Malatapy, especially to the family of Benedicto "Juan" Alaton.

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Appendix. - Ichthyofauna of the seagrass meadow in Malatapay, Negros Oriental, Philippines (\* = species not identified).

Family Species		Beach seine								Visual census technique					
		Pooled day and night samples				Pooled stations				Size range	Distance to coral reef				Size range
		Distance to coral reef				Day		Night			Distance to coral reef				
		50 m		250 m							50 m		250 m		
		n	%	n	%	n	%	n	%		TL (cm)	n	%	n	
1. Acanthuridae	12	0.1	8	0.1			20	0.2		34	0.5	23	0.7		
1. <i>Naso</i> sp.	12	0.1	8	0.1			20	0.2	3.3-5.8	34	0.5	23	0.7	4.0-7.0	
2. Antennariidae	1	< 0.1					1	< 0.1							
2. <i>Antennarius commersoni</i>	1	< 0.1					1	< 0.1	3.3						
3. Apogonidae	4398	54.5	1668	23.5	791	15.6	5275	52.1		24	0.4	1202	36.2		
3. <i>Apogon aureus</i>	24	0.3	145	2			169	1.7	5.1-11.9						
4. <i>Apogon cyanosoma</i>	2185	27.1	35	0.5	21	0.4	2199	21.7	1.0-6.8						
5. <i>Apogon</i> sp.	261	3.2	33	0.5	4	< 0.1	290	2.9	1.1-6.3						
6. Apogonidae*	1551	19.2	1214	17.1	513	10.2	2252	22.2	0.9-18.5	24	0.4	1202	36.2	2.0	
7. <i>Cheilodipterus quinquelineatus</i>	377	4.7	241	3.4	253	5	365	3.6	0.6-11.3						
4. Belonidae										1	< 0.1				
8. <i>Tylosurus crocodilus crocodilus</i>										1	< 0.1			50.0	
5. Blenniidae			1	< 0.1			1	< 0.1		5	< 0.1				
9. <i>Meiacanthus grammistes</i>										5	< 0.1			8.0-10.0	
10. <i>Nemophini</i> *			1	< 0.1			1	< 0.1	4.2						
6. Bothidae	224	2.7	394	5.6	268	5.3	350	3.4		1	< 0.1	1	< 0.1		
11. Bothidae*	204	2.5	387	5.5	249	4.9	342	3.4	1.0-7.8						
12. <i>Bothus pantherinus</i>	20	0.2	7	0.1	19	0.4	8	< 0.1	4.2-18.1	1	< 0.1	1	< 0.1		
7. Caesionidae	1	< 0.1					1	< 0.1				4	0.1		
13. <i>Caesio</i> sp.												4	0.1	25.0	
14. <i>Pterocaesio pisang</i>	1	< 0.1					1	< 0.1	4.8						
8. Chaetodontidae												3	0.1		
15. <i>Chaetodon</i> sp.												3	0.1	4.0	
9. Callionymidae	10	0.1	13	0.2	9	0.2	14	0.1							
16. Callionymidae*	10	0.1	12	0.2	8	0.2	14	0.1	1.8-13.7						
17. <i>Dactylopus dactylopus</i>			1	< 0.1	1	< 0.1			17						
10. Carangidae			1	< 0.1	1	< 0.1									
18. <i>Carangoides</i> sp.			1	< 0.1	1	< 0.1			9.6						

<b>11. Carapidae</b>	1	< 0.1					1	< 0.1						
19. Carapidae*	1	< 0.1					1	< 0.1	10.4					
<b>12. Congridae</b>	12	< 0.1	7	< 0.1			19	0.1						
20. <i>Conger cinereus</i>	7	< 0.1	5	< 0.1			12	0.1	8.8-26.3					
21. <i>Conger</i> sp.	1	< 0.1					1	< 0.1	19.2					
<b>22. Congridae*</b>	4	< 0.1	2	< 0.1			6	< 0.1	1.8-24.1					
<b>13. Cynoglossidae</b>	2	< 0.1	3	< 0.1	1	< 0.1	4	< 0.1						
23. Cynoglossidae*	2	< 0.1	3	< 0.1	1	< 0.1	4	< 0.1	7.4-14.9					
<b>14. Dasyatidae</b>			2	< 0.1	2	< 0.1								
24. <i>Taeniura lymna</i>			2	< 0.1	2	< 0.1			39.0-40.5					
<b>15. Diodontidae</b>	1	< 0.1	37	0.5	11	0.2	27	0.3			1	< 0.1		
25. <i>Diodon holocanthus</i>	1	< 0.1	37	0.5	11	0.2	27	0.3	10.4-21.5		1	< 0.1		
<b>16. Fistulariidae</b>			1	< 0.1			1	< 0.1						
26. <i>Fistularia commersonii</i>			1	< 0.1			1	< 0.1	14.2					
<b>17. Gobiidae</b>	61	0.6	237	3.3	75	1.4	223	2.2		15	0.2	32	1.0	
27. <i>Amblygobius phalaena</i>			1	< 0.1	1	< 0.1			5.8					
28. <i>Amblygobius sphynx</i>	6	< 0.1	74	1	32	0.6	48	0.5	1.1-7.3					
29. Gobiidae*	29	0.4	161	2.3	17	0.3	173	1.7	1.0-6.4	8	0.1	26	0.8	1.0-7.0
30. <i>Valenciennea longipinnis</i>	1	< 0.1			1	< 0.1			5.5					
31. <i>Valenciennea puellaris</i>	7	< 0.1			6	0.1	1	< 0.1	6.2-12.2	5	0.1	4	0.1	5.0-8.0
32. <i>Valenciennea sexguttata</i>	18	0.2	1	< 0.1	18	0.4	1	< 0.1	2.2-7.9	2	< 0.1	2	< 0.1	8.0-10.0
<b>18. Haemulidae</b>	7	< 0.1	2	< 0.1	6	0.1	3	< 0.1						
33. <i>Plectorhinchus diagrammus</i>	7	< 0.1	2	< 0.1	6	0.1	3	< 0.1	1.6-4.4					
<b>19. Holocentridae</b>	17	2.4					17	0.2						
34. Holocentridae*	17	2.4					17	0.2	2.7-7.9					
<b>20. Labridae</b>	1053	12.8	2297	32.1	2524	49.8	826	8.2		1056	16.8	403	12.1	
35. <i>Cheilinus bimaculatus</i>	411	5.1	1861	26.2	1514	30.1	758	7.5	0.7-12.9	65	1.0	74	2.2	5.0-15.0
36. <i>Cheilinus trilobatus</i>	16	0.2	51	0.7	37	0.7	30	0.3	2.5-10.1			4	0.1	10.0-12.0
37. <i>Cheilio inermis</i>	277	3.4	208	2.9	448	8.9	37	0.4	1.2-21.6	180	2.9	121	3.6	10.0-35.0
38. <i>Halichoeres chloropterus</i>	82	1	75	1.1	157	3.1			1.1-8.4	101	1.6	103	3.1	1.0-6.0
39. <i>Halichoeres interrupta</i>			1	< 0.1	1	< 0.1			3.8					
40. <i>Halichoeres margaritaceus</i>	1	< 0.1	6	< 0.1	1	< 0.1			3.8					
41. <i>Halichoeres miniatus</i>	1	< 0.1			1	< 0.1			2.7					
42. <i>Halichoeres scapularis</i>	49	0.6			55	1.1			1.1-4.3	182	2.9	77	2.3	3.0-20.0
43. <i>Halichoeres</i> sp.	32	0.4	15	0.2	47	0.9			1.5-8			3	0.1	5.0-8.0
44. <i>Halichoeres</i> : zeylonicus	6	< 0.1	2	< 0.1	7	0.1	1	< 0.1	2.1-11.5	1	< 0.1			15.0
45. Labridae*	21	0.3	16	0.2	37	0.7			1.2-7.1	188	3.0	17	0.5	3.0-10.0
46. <i>Novaculichthys macrolepidotus</i>	59	0.7	29	0.4	88	1.7			5.5-16.2	4	< 0.1	4	0.1	10.0-12.0

Family Species	Beach seine									Visual census technique				
	Pooled day and night samples				Pooled stations				Size range	Distance to coral reef				Size range
	Distance to coral reef				Day		Night			Distance to coral reef				
	50 m		250 m							50 m		250 m		
	n	%	n	%	n	%	n	%	TL (cm)	n	%	n	%	TL (cm)
47. <i>Pteragogus flagellifer</i>			3	< 0.1	3	< 0.1			7.3-8.7					
48. <i>Stethojulis interrupta</i>	89	1.1	30	0.4	119	2.4			1.0-11.9					
49. <i>Thalassoma lunare</i>	7	< 0.1			7	0.1			8.6-17.3	335	5.3			10.0-15.0
50. <i>Xyrichtys pentadactylus</i>	2	< 0.1			2	< 0.1			15.2-15.7					
21. <b>Lethrinidae</b>	69	0.9	583	8.2	399	7.9	253	2.5		32	0.5	5	0.2	
51. <i>Lethrinidae*</i>	69	0.9	582	8.2	398	7.9	253	2.5	1.1-6.8					
52. <i>Lethrinus genivittatus</i>			1	< 0.1	1	< 0.1			9.5					
53. <i>Lethrinus harak</i>										32	0.5	5	0.2	18.0-40.0
22. <b>Lutjanidae</b>	2	< 0.1	7	< 0.1	2	< 0.1	7	< 0.1						
54. <i>Lutjanidae*</i>			2	< 0.1			2	< 0.1	1.9-2.5	1	< 0.1			
55. <i>Lutjanus fluviiflamma</i>	2	< 0.1	4	< 0.1	2	< 0.1	4	< 0.1	2.2-19.7	1	< 0.1			
56. <i>Lutjanus kasmira</i>			1	< 0.1			1	< 0.1	9.3					
23. <b>Monacanthidae</b>	3	< 0.1	7	0.1	5	< 0.1	5	< 0.1		5	0.1	1	< 0.1	
57. <i>Monacanthidae*</i>	3	< 0.1	7	0.1	5	< 0.1	5	< 0.1	1.2-12.4	5	0.1	1	< 0.1	4.0
24. <b>Mugilidae</b>												2	< 0.1	
58. <i>Crenimugil crenilabis</i>													2	< 0.1
25. <b>Mullidae</b>	60	0.6	224	3.2	213	4.1	71	0.4		42	0.7	134	4.0	
59. <i>Mullidae*</i>										19	0.3	9	0.3	4.0-6.0
60. <i>Mulloidichthys flavolineatus</i>	10	0.1					10	< 0.1	7.7-8.8					
61. <i>Parupeneus barberinoides</i>	16	0.2	23	0.3	33	0.7	6	< 0.1	3.3-8.2					
62. <i>Parupeneus barberinus</i>	28	0.3	169	2.4	163	3.2	34	0.3	3.3-7.8			79	2.4	4.0-10.0
63. <i>Parupeneus cyclostomus</i>	1	< 0.1	20	0.3	7	0.1	14	0.1	6.5-14.2					
64. <i>Parupeneus indicus</i>			1	< 0.1			1	< 0.1	5.8	6	0.1	29	0.9	7.0-35.0
65. <i>Parupeneus multifasciatus</i>	3	< 0.1			3	< 0.1			5.4-7.6	15	0.2	2	< 0.1	4.0
66. <i>Upeneus tragula</i>	2	< 0.1	11	0.2	7	0.1	6	< 0.1	2.9-17.1	2	< 0.1	15	0.5	4.0-18.0
26. <b>Muraenidae</b>	2	< 0.1	5	< 0.1			7	< 0.1		3	< 0.1	1	< 0.1	
67. <i>Enchelynassa canina</i>			2	< 0.1			2	< 0.1	26.1-35.5					
68. <i>Gymnothorax chilospilus</i>	1	< 0.1	2	< 0.1			3	< 0.1	23.4-30.0					
69. <i>Gymnothorax pseudothyrsoides</i>	1	< 0.1	1	< 0.1			2	< 0.1	34.0-34.6					
70. <i>Muraenidae*</i>										3	< 0.1	1	< 0.1	20.0-40.0

<b>27. Nemipteridae</b>	5	< 0.1	19	0.2	9	0.1	15	0.1					
71. Nemipteridae*	1	< 0.1	5	< 0.1	6	0.1			2.0-2.1				
72. <i>Scolopsis affinis</i>			14	0.2	1	< 0.1	13	0.1	9.3-13.1				
73. <i>Scolopsis bilineatus</i>	3	< 0.1			2	< 0.1	1	< 0.1	3.0-7.2				
74. <i>Scolopsis</i> sp.	1	< 0.1					1	< 0.1	7.8				
<b>28. Ophichthidae</b>	3	< 0.1	5	< 0.1	1	< 0.1	7	< 0.1					
75. <i>Leiuranus semicinctus</i>			1	< 0.1			1	< 0.1	50.5				
76. <i>Muraenichthys macropterus</i>	3	< 0.1	4	< 0.1	1	< 0.1	6	< 0.1	9.6-18.4				
<b>29. Ostraciidae</b>			2	< 0.1	1	< 0.1	1	< 0.1					
77. <i>Ostracion cubicus</i>			2	< 0.1	1	< 0.1	1	< 0.1	1.2-1.9				
<b>30. Pinguipedidae</b>	5	< 0.1	1	< 0.1	3	< 0.1	3	< 0.1		19	0.3	4	0.1
78. <i>Parapercis cylindrica</i>	5	< 0.1	1	< 0.1	3	< 0.1	3	< 0.1	2.7-10.7				
79. <i>Parapercis</i> sp.										19	0.3	4	0.1
<b>31. Platycephalidae</b>	6	< 0.1	9	< 0.1	3	< 0.1	12	< 0.1					5.0-12.0
80. Platycephalidae*	3	< 0.1	6	< 0.1	1	< 0.1	8	< 0.1	2.1-12.6				
81. <i>Thysanophrys arenicola</i>	3	< 0.1	3	< 0.1	2	< 0.1	4	< 0.1	4.1-10.5				
<b>32. Plotosidae</b>	1276	15.8	227	3.2			1503	14.8		1060	17.0	501	15.1
82. <i>Plotosus lineatus</i>	1276	15.8	227	3.2			1503	14.8	1.3-25.5	1060	17.0	501	15.1
<b>33. Pomacentridae</b>	72	0.8	24	0.2	90	1.7	6			3008	47.9	163	4.9
83. <i>Abudefduf vaigiensis</i>			15	0.2	13	0.3	2	< 0.1	3.0-5.6				
84. <i>Amphiprion clarki</i>	12	< 0.1	2	< 0.1	3	< 0.1	1	< 0.1	2.6-7.5	61	0.9	3	0.1
85. <i>Amphiprion polymnus</i>			1	< 0.1	1	< 0.1			6.9				3.0-12.0
86. <i>Amphiprion</i> sp.										8	0.1	2	0.1
87. <i>Chromis</i> sp.										1	< 0.1		2.0-10.0
88. <i>Dascyllus</i> sp.	1	< 0.1			1	< 0.1			10.1			1	8.0
89. <i>Dascyllus trimaculatus</i>	6	< 0.1	2	< 0.1	6	0.1	2	< 0.1	1.7-10.6	19	0.3		< 0.1
90. <i>Pomacentrus coelestis</i>	63	0.8	4	< 0.1	66	1.3	1	< 0.1	1.5-7.6	2809	44.8	50	1.5
91. Pomacentridae*										110	1.8	107	3.2
<b>34. Scaridae</b>	232	2.8	240	3.3	365	7.1	107	1		45	0.7	52	1.6
92. <i>Calotomus spinidens</i>	218	2.7	237	3.3	350	6.9	105	1	0.9-15.9				
93. <i>Leptoscarus vaigiensis</i>	10	0.1	2	< 0.1	10	0.2	2	< 0.1	1.9-18.5				
94. Scaridae*	4	< 0.1	1	< 0.1	5	< 0.1			1.5-2.4	45	0.7	52	1.6
<b>35. Scorpaenidae</b>	328	4	509	7.1	22	0.3	815	8					5.0-25.0
95. <i>Ablabys taenianotus</i>	8	0.1	7	0.1	4	< 0.1	11	0.1	3.1-9.7				
96. <i>Dendrochirus brachypterus</i>			2	< 0.1	2	< 0.1			10.6-10.7				
97. <i>Dendrochirus zebra</i>			2	< 0.1	2	< 0.1			8.5-10.5				
98. <i>Inimicus caledonicus</i>			1	< 0.1			1	< 0.1	9.6				

Family Species		Beach seine								Visual census technique					
		Pooled day and night samples				Pooled stations				Size range TL (cm)	Distance to coral reef				Size range TL (cm)
		Distance to coral reef				Day		Night			Distance to coral reef				
		50 m		250 m							50 m		250 m		
		n	%	n	%						n	%	n	%	
99. <i>Paracentropogon longispinus</i>	1	< 0.1					1	< 0.1	2.7						
100. <i>Parascorpaena mossambica</i>			1	< 0.1			1	< 0.1	9.5						
101. <i>Scorpaenodes</i> sp.	319	3.9	496	7	14	0.3	801	7.9	0.9-11.8						
36. <b>Siganidae</b>	68	0.7	399	5.7	167	3.3	300	2.9		917	14.6	790	23.7		
102. <i>Siganus canaliculatus</i>	6	< 0.1	12	0.2	2	< 0.1	16	0.2	11.0-17.0						
103. <i>Siganus</i> sp.	55	0.7	387	5.5	165	3.3	277	2.7	0.8-4.7	917	14.6	790	23.7	10.0-20.0	
104. <i>Siganus spinus</i>	7	< 0.1					7	< 0.1	10.5-15.0						
37. <b>Sillaginidae</b>	1	< 0.1					1	< 0.1							
105. <i>Sillago sihama</i>	1	< 0.1					1	< 0.1	9.8						
38. <b>Soleidae</b>	22	0.2	12	0.2	4		30	2.5							
106. <i>Parduchirus pavoninus</i>	6	< 0.1	1	< 0.1	2	< 0.1	5	< 0.1	4.2-14.5						
107. <i>Soleidae</i> *	16	0.2	11	0.2	2	< 0.1	25	2.5	1.9-11.0						
39. <b>Sphyraenidae</b>	51	0.6	104	1.5	6	0.1	149	1.5							
108. <i>Sphyraena flavicauda</i>	42	0.5	97	1.4	6	0.1	133	1.3	2.7-11.2						
109. <i>Sphyraena</i> sp.	9	0.1	7	0.1			16	0.2	4.0-5.5						
40. <b>Syngnathidae</b>	10	< 0.1			6	< 0.1	4	< 0.1							
110. <i>Doryrhamphus dactyliophorus</i>	3	< 0.1			2	< 0.1	1	< 0.1	7.1-35.9						
111. <i>Micrognathus vittatus</i>	1	< 0.1					1	< 0.1	2.5						
112. <i>Syngnathidae</i> sp.	3	< 0.1			1	< 0.1	2	< 0.1	15.5-19.6						
113. <i>Trachyrhamphus bicourctatus</i>	3	< 0.1			3	< 0.1			7.5-33.4						
41. <b>Synodontidae</b>	75	0.8	32	0.5	41	0.6	66	0.5		3	< 0.1				
114. <i>Saurida gracilis</i>	24	0.3	13	0.2	17	0.3	20	0.2	3.2-16.5	3	< 0.1			15.0-18.0	
115. <i>Synodontidae</i> *	41	0.5	12	0.2	18	0.3	35	0.3	1.2-4.0						
116. <i>Synodus dermatogenys</i>	4	< 0.1			3	< 0.1	1	< 0.1	3.0-4.1						
117. <i>Trachinocephalus myops</i>	6	< 0.1	7	0.1	3	< 0.1	10	< 0.1	2.5-9.9						
42. <b>Tetraodontidae</b>	3	< 0.1	2	< 0.1	1	< 0.1	4	< 0.1				1	< 0.1		
118. <i>Arothron</i> sp.	1	< 0.1					1	< 0.1	31.2			1	< 0.1	30.0	
119. <i>Arothron stellatus</i>		1	< 0.1			1	< 0.1	56.5							
120. <i>Canthigaster hennetti</i>	2	< 0.1			1	< 0.1	1	< 0.1	3.3-7.7						
121. <i>Tetraodontidae</i> *			1	< 0.1			1	< 0.1	2.4						
<b>Total</b>	8,087	100	7,078	100	5,026	100	10,139	100		6,276	100.0	3,324	100.0		



## TEMPORAL PATTERNS OF SETTLEMENT OF THREE SPECIES OF DAMSELFISH OF THE GENUS *DASCYLLUS* (POMACENTRIDAE) IN THE CORAL REEFS OF FRENCH POLYNESIA

by

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**ABSTRACT.** - Patterns of settlement from the plankton of three damselfish species in the genus *Dascyllus* were estimated at the island of Moorea, French Polynesia, for three temporal scales: among seasons of the year, within a lunar month, and among days. The three species (*D. trimaculatus*, *D. flavicaudus*, and *D. aruanus*) displayed qualitatively similar settlement patterns at all scales examined. There was distinct seasonality with the greatest settlement occurring in the Austral winter months of June-August. Within a lunar month, fish primarily settled around the two quarter moon phases, with substantially lower settlement occurring near periods of full and new moon. Daily estimates of larval settlement revealed distinct peaks, typically lasting 3-5 days, with little settlement during intervening days. The mean interval between settlement peaks was 14 days, resulting in an average of two pulses of settlement each lunar month. The timing of peak settlement during a colonization cycle was quite similar among the damselfishes, but was not constant with respect to the actual date of the quarter moon. For all three species, the intensity of settlement (density of new colonists) varied among cycles by about 1.5 orders of magnitude. *D. trimaculatus* and *D. flavicaudus* showed high temporal concordance in the intensity of settlement among cycles, whereas variation among cycles in the settlement strength of *D. aruanus* was uncorrelated with either *D. trimaculatus* or *D. flavicaudus*.

**RÉSUMÉ.** - Chronologie de l'installation de trois espèces de poissons-demoiselles du genre *Dascyllus* (Pomacentridae) sur les récifs coralliens de Polynésie française.

Nous avons étudié l'installation, à partir des larves planctoniques, de trois espèces de poissons-demoiselles du genre *Dascyllus* sur l'Île de Moorea, en Polynésie Française et selon trois échelles de temps: la saison, le cycle lunaire et d'un jour à l'autre. Qualitativement les trois espèces (*D. trimaculatus*, *D. flavicaudus* et *D. aruanus*) présentent des modèles semblables d'installation à tous les niveaux et à toutes les échelles. En ce qui concerne la saisonnalité, l'installation est la plus importante de juin à août, durant l'hiver austral. Pour le cycle lunaire, l'installation est la plus importante lors des quartiers de lune, avec les diminutions d'intensité lors des pleine et nouvelle lunes. Les estimations quotidiennes des intensités d'installation montrent tous les 14 jours un pic d'une durée de 3 à 5 jours environ, soit deux pics d'installation chaque cycle lunaire. Durant un cycle de colonisation, ces pics semblent relativement constants chez ces poissons-demoiselles et ne semblent pas être directement corrélés aux cycles des quartiers de lune. Pour les trois espèces étudiées, l'intensité de l'installation (densité des nouveaux arrivants) varie d'un facteur 1,5 entre les différents cycles. Les espèces *D. trimaculatus* et *D. flavicaudus* montrent une grande concordance temporelle dans l'intensité de leur installation au cours des différents cycles étudiés; il n'en est pas de même entre chacune d'entre-elles et *D. aruanus*.

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Key-words. - Pomacentridae, *Dascyllus aruanus*, *Dascyllus flavicaudus*, *Dascyllus trimaculatus*, ISE, French Polynesia, Coral reef fishes, Recruitment, Settlement, Lunar periodicity, Population dynamics, Seasonality.

The replenishment of populations of most coral reef fishes is via a planktonic larval stage that disperses from the parental population before settling to the reef environment. Despite the centrally important role of such replenishment to the population dynamics of coral reef fishes, major gaps exist in our basic understanding of temporal patterns in larval settlement. While it is clear that variation in settlement of coral reef fishes has been found at every temporal scale examined (Williams, 1983), relatively few generalities have emerged. Among the few, seemingly universal features are that settlement of coral reef fishes almost always occurs during the night rather than during daylight hours (Doherty and Williams, 1988; Booth, 1991), and that settlement typically is lower during the full moon period than at other times of the lunar month (Sale, 1985; Doherty and Williams, 1988; Dufour *et al.*, 1996). Presumably these patterns reflect evolved adaptations that reduce mortality of highly vulnerable young fishes from visual predators (e.g., Sale, 1985; Doherty and Williams, 1988; Doherty, 1990; Dufour and Galzin, 1993). However, there appears to be considerable variation among species and/or locations in the existence or extent of seasonality, in the existence or type of lunar periodicity, and in the pattern of variation in the magnitude of settlement of a species among different colonization episodes. For example, MacFarland *et al.* (1985) found that the French grunt *Haemulon flavolineatum* in the Caribbean settled monthly near the new moon in discrete peaks that were of similar magnitude throughout the year, whereas Booth (1992) reported that the damselfish *Dascyllus albisella* settled in Hawaii in two episodes, each spread over several weeks, only during summer months without any clear indication of lunar periodicity. Until more systematic studies are conducted, it is not possible to determine how much of the observed variation in such temporal patterns of settlement can be attributed to different locations (e.g., the Caribbean vs. Hawaii), differences among taxonomic groups of species (e.g., grunts vs. damselfishes) and/or differences among similar species (e.g., among damselfishes).

Among the most comprehensive studies of temporal patterns in the colonization of an assemblage of coral reef fishes are those of Dufour and Galzin (1993), Dufour *et al.* (1996) and Planes *et al.* (1993) for the island of Moorea, French Polynesia. Dufour and co-workers used stationary nets at the crest of the barrier reef to estimate nightly the flux of late stage larvae that entered lagoons; at all sites and times sampled, the highest fluxes occurred near periods of new moon. The larvae of some taxa, however, appeared to enter the lagoon most abundantly near quarter moon phases (Dufour *et al.*, 1996). This stationary crest net approach, which subsequently has been used to estimate larval flux rates to coral reefs in eastern and western Australia (Doherty and McIlwain, 1996), has several advantages and drawbacks. It provides a means to compare concurrent patterns of larval colonization across many species and taxa of fishes. However, the approach is highly labor intensive, potential sampling biases (among species or taxa) are difficult to assess, and analysis of patterns at the species level is limited to the relatively few species that are caught in sufficiently high abundance. For example, only one species of damselfish (*Stegastes nigricans*) was trapped in sufficient numbers by Dufour *et al.* (1996) to allow analyses of temporal patterns, despite the fact that damselfishes are among the most



numerous (by species and abundance) reef fishes in the lagoons of Moorea (Galzin, 1987a, 1987b).

We take a complementary approach to that used by Dufour and Galzin (1993), Dufour *et al.* (1996) and Doherty and McIlwain (1996) to examine temporal patterns of settlement of three closely related species of damselfishes. Our approach was to census daily the number of new settlers that colonized a standard amount of settlement microhabitat, and to use that technique to examine temporal patterns and coherence among the species at a series of scales (i.e., seasonal, lunar, daily) in the same location (the north shore of Moorea, French Polynesia). Other workers have estimated settlement from daily counts of new colonists (Booth, 1991; Robertson, 1992), although more typically the interval between successive samples has been considerably longer than a day (e.g., Milicich *et al.*, 1992; Meekan *et al.*, 1993; Planes *et al.*, 1993; Sponaugle and Cowen, 1994, 1996, 1997). Further, the time frame over which observations are made typically has been relatively short (e.g., ~2 lunar months) and the number of species examined concurrently usually has been quite low (for an exception, see Planes *et al.*, 1993). This general approach, however, has the advantage of providing a more accurate estimation of actual settlement patterns, especially when estimates are made daily, microhabitat associations are known and care is taken to reduce the influence of older conspecifics and other species (Booth, 1991; Robertson, 1992).

Here we present data on concurrent patterns of settlement of the damselfishes at three temporal scales: among seasons of the year, within a lunar month, and among days. The species of damselfishes we examined were the Three-spot dascyllus (*Dascyllus trimaculatus*), the Yellow-tailed dascyllus (*D. flavicaudus*), and the Humbug dascyllus (*D. aruanus*). Like all damselfishes, these species lay benthic eggs that are fertilized externally. Larvae hatch and enter the plankton after ~3 days (Garnaud, 1957; Thresher, 1984), and settle back to the reef environment after a planktonic duration that averages 22-24 days (Brothers *et al.*, 1983; Wellington and Victor, 1989). *D. trimaculatus* settle to sea anemones (Dunn, 1981; Fautin, 1985; Fautin and Allen, 1992) where they remain throughout the juvenile phase; adults are free-living and do not associate closely with anemones for shelter. By contrast, *D. aruanus* settle to corals and remain associated with them throughout their lives (Sale, 1971; Forrester, 1990; Allen, 1991). *D. flavicaudus* tend to retain some degree of microhabitat association as they age. In Moorea, the most common species of anemone (*Heteractis magnifica*) is occupied by *D. trimaculatus*, whereas young *D. aruanus* and *D. flavicaudus* primarily settle to heads of live *Pocillopora* (primarily *P. meandrina* and *P. eydouxi*; hereafter *Pocillopora*); *D. flavicaudus* also settle to plating *Acropora* and certain species of *Montipora*.

## MATERIALS AND METHODS

All field work reported here was conducted in lagoons on the north shore of Moorea (17°30'S, 149°50'W) in French Polynesia. A barrier reef encircles the triangular-shaped island, forming a system of lagoons that average ~1 km in width and ~5 m in water depth (for more details, see Galzin and Pointier, 1985). The bottom within the lagoons is composed of patch reefs that are interspersed with unconsolidated sediments. On the north shore, the barrier reef is cut by two deep bays (Cooks and Opunohu) and three smaller passes. Water primarily enters the lagoons over the crest of the barrier reef and exits through the passes. It should be noted that the tidal amplitude at Moorea is quite

small (~50 cm) with little direct influence of the moon as the island is close to an amphidromic point (Bongers and Wyrki, 1987). As a consequence, water flow within the lagoon appears to be influenced principally by offshore swell conditions.

To estimate settlement, we transplanted settlement microhabitat (anemones and corals) to areas that previously lacked such habitat. Forty anemones (*Heteractis magnifica*) were transplanted at depths of 4-6 m to a fringing reef on the eastern flank of the Vaipahu Lagoon adjacent to the Gump South Pacific Biological Station, and forty live heads of *Pocillopora* at 3 m depth on an expanse of sand approximately 0.5 km north-west of the anemone transplant site (for more complete description of the study sites, see Schmitt and Holbrook, 1996). Corals were affixed individually to cinder blocks using Z-Spar Splash Zone Compound®, while anemones were allowed to attach naturally on the fringing reef or a cinder block. Each settlement microhabitat was approximately 5 m from its nearest neighbor, and a minimum of 25 m from naturally occurring anemones or live heads of coral.

Unless noted otherwise, estimates of settlement were obtained from daily counts of fish in all settlement microhabitats (i.e., transplanted anemones and corals). New settlers, which arrived at night, are distinguished from individuals that settled on previous days by the lack of pigmentation of the tail and peduncle (Schmitt and Holbrook, 1996; Holbrook and Schmitt, 1997). At the initiation of observations, resident fish were removed from the microhabitats as resident conspecifics can influence the settlement of *Dascyllus* (e.g., Sweatman, 1983, 1985; Sweatman and St. John, 1990; Schmitt and Holbrook, 1996). Fish that accumulated were removed at least every two weeks during extended periods of observation. As settlement tended to occur in 14-day cycles (see Results), we estimated the number of new settlers over periods of two weeks, each of which encompassed the period of maximum settlement. The location and total area of microhabitat sampled each date remained constant; data on *D. trimaculatus* settlers are expressed as the number per 40 anemones (each with a diameter of ~30 cm), whereas those for the other two species are given as the number per 40 heads of *Pocillopora* (each was ~30 cm in diameter and 20 cm tall).

To examine seasonality, the calendar year was divided into four seasons representing the Austral winter (June-August), spring (September-November), summer (December-February) and fall (March-May). For each season, the cumulative number of *Dascyllus* that settled to the transplanted settlement microhabitats in 'replicate' two-week cycles was counted on the focal anemones and corals. For each season, cycles were counted in two (fall) or three (all other seasons) different years (1995-1997), with the 'replicate' number of cycles being 12 (winter), 5 (spring and summer) and 3 (fall). For each species and season, the mean density of settlers (cumulative total in two weeks per 40 settlement microhabitats) was calculated and difference among seasons tested by ANOVA.

Possible lunar patterns in settlement were examined for 9 (*D. trimaculatus*) or 6 (remaining 2 species) lunar months during periods of heavy settlement in 1995-1997. For four of the lunar months used in this analysis, estimates of settlement for *D. flavicaudus* and *D. aruanus* to corals were made approximately every other day (rather than daily) on the 40 transplanted corals; settlement of *D. trimaculatus* was estimated daily to the transplanted anemones for all lunar months. The lunar month was divided into four equal periods (weeks), with a week centered on the time of new moon, the first quarter, full moon or the third quarter. Since the absolute number of settlers varied considerably among the species and among the lunar months, we standardized the data to facilitate comparisons. This was done by dividing the number of settlers of a given species that arrived during

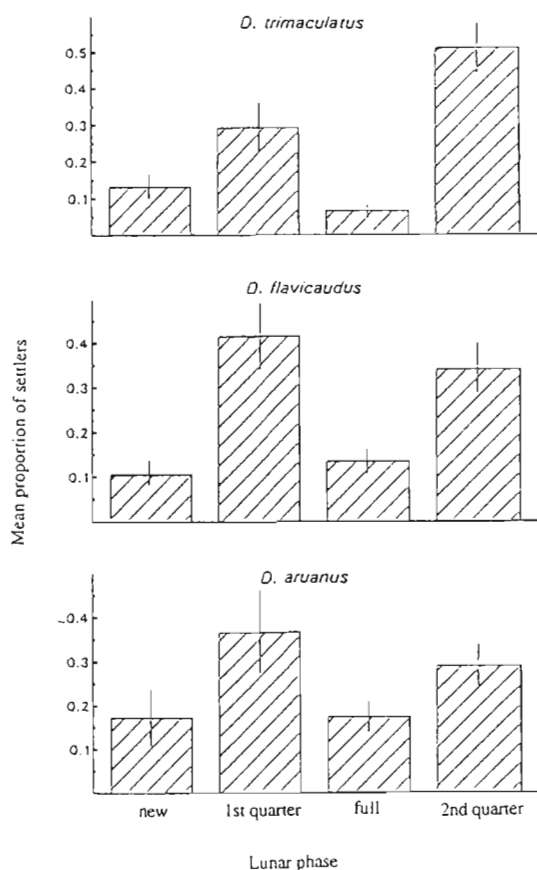


Fig. 1. - Seasonality in settlement of the 3 species of *Dascyllus* at Moorea, French Polynesia. Data are the mean (1 SE) number of settlers that colonized 40 initially empty settlement microhabitats (anemones or coral heads) in replicate 14-day periods, each of which encompassed a quarter moon and a new or full moon period. Seasons correspond to the months of March-May (fall;  $n = 3$  replicate periods), June-August (winter;  $n = 12$ ), September-November (spring;  $n = 5$ ), and December-February (summer;  $n = 5$ ).

each of the four weeks by the total number that colonized during that lunar month. For each species, the mean proportion (and standard error) of settlers that arrived was calculated for each of the four moon phases.

We had a sufficiently long record of daily settlement estimates of *D. trimaculatus* to perform time-series analyses. To determine whether cycles existed and estimate the periodicity in settlement, autocorrelations among different lag (day) intervals were calculated using an ARIMA (autoregressive integrated moving-average) model. Lag lengths up to 60 days were calculated using  $\log_{10}(\text{settler density} + 1)$  transformed data. (Using log transformed data not only resolved the inherent problem that variance scales positively with the mean for density estimates, in the present application it also facilitated detection of cycle periodicities by reducing the influence of especially large settlement peaks.)

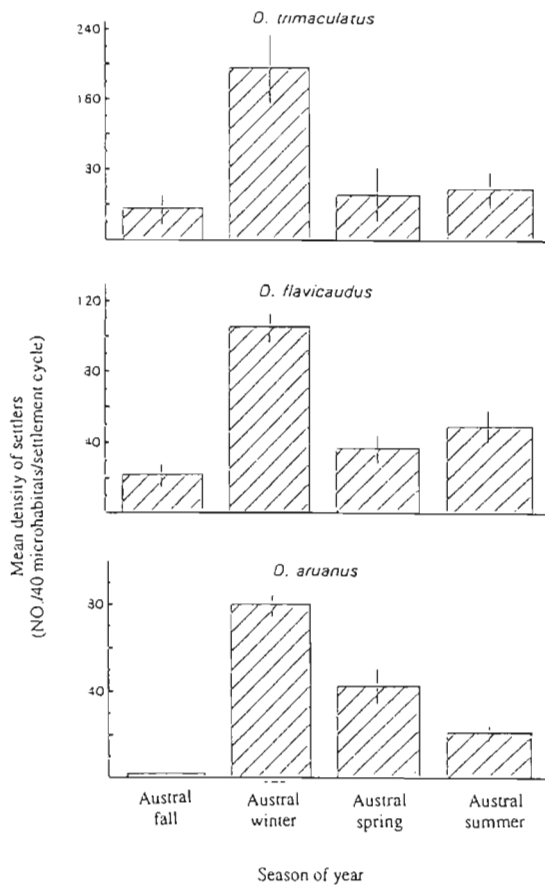


Fig. 2. - Lunar patterns of settlement of three *Dascyllus* species at Moorea, French Polynesia. Data are the mean (1 SE) proportion of settlers in a lunar month that colonized during each of the 4-week periods that represent the different moon phases. The number of lunar months examined was 9 for *D. trimaculatus* and 6 for the other two species.

Concordance among the three damselfish species in the timing of colonization was examined for five two-week periods when daily counts of all species were made concurrently and when species settled in relatively high numbers. To facilitate comparisons in timing among species and settlement episodes, data for each species and 14-day settlement event were standardized by dividing the number of settlers each day by the total that arrived over that 14-day period.

Temporal concordance among the species in the intensity of settlement (i.e., total density of colonists) was examined using 15 two-week long periods when we had estimates for all three species. Data for 9 of these periods were obtained from counts of the forty anemones and corals used for the analyses described above. Data for the remaining six two-week periods were obtained from counts of settlers to thirty additional anemones and corals that were transplanted to other lagoon locations on the north shore of Moorea using techniques described previously. These additional sites were located halfway between the barrier reef and shore off the villages of Maharepa and Papetoai. For two of the

six periods, counts of the thirty additional microhabitats were made daily; for the remaining four periods, settlement intensities were estimated from counts made bi-weekly (3-4 days after peak settlement). Correlations in the total density of settlers (number per standardized area of microhabitat) were calculated for the three pairwise combinations of damselfish species.

## RESULTS

Although all three species of *Dascyllus* settled throughout the year at Moorea, there was a marked pattern of seasonality (Fig. 1). For all species, the average density of new colonists that arrived in a standardized settlement episode (i.e., a 14-day period that encompassed peak colonization) differed statistically among the seasons ( $p < 0.05$  for all ANOVAs). The period of greatest settlement for each species of *Dascyllus* occurred during the Austral winter months of June-August when the average density of colonists per settlement event was 2-4 times greater than the season with the next highest settlement rate (Fig. 1). Within a species, levels of settlement were generally similar among the three non-winter seasons, although *D. aruanus* appeared to have especially low settlement during the Austral fall period of September-December. It should be noted, however, that our estimates for the Austral fall were based on substantially fewer measurements than for other periods of the year (see Materials and Methods). Nonetheless, the data do support the conclusion that settlement of these *Dascyllus* at Moorea was seasonal and that all three species showed the same qualitative pattern at this temporal scale.

With respect to settlement at the scale of a lunar month, colonization from the plankton of each *Dascyllus* species was non-random among the four lunar weeks. While larvae of each species settled during all phases of the lunar month, most colonized during the weeks of the two quarter moon phases (Fig. 2). *D. trimaculatus* showed the greatest disparity between quarter moon and full and new moon periods, whereas *D. aruanus* showed the smallest difference. On average, 80% of the *D. trimaculatus* colonizers that arrived during a lunar month settled during the quarter moon periods, compared with 76% for *D. flavicaudus* and 66% for *D. aruanus* (Fig. 2;  $p < 0.05$  for each test of uniform settlement). With the possible exception of *D. trimaculatus*, there was little difference in the proportion of new colonists that arrived during new and full moon periods (Fig. 2). The average proportion of *D. trimaculatus* that settled during the week of full moon (6.6%) was half that during the new moon period (13.1%), although the difference was not statistically significant ( $p = 0.12$ ).

We had the longest time series of daily settlement for *Dascyllus trimaculatus*, which revealed that the temporal pattern of colonization was more complex than was suggested by the analyses above. In general, daily settlement of *D. trimaculatus* over the course of several lunar months was episodic, showing distinct peaks with relatively little or no settlement during intervening days (Fig. 3). The cyclic nature of settlement was indicated by positive autocorrelation in the density of *D. trimaculatus* settlers at about 14-day intervals (i.e., lags of ~14, ~28, ~42, ~56 days; Fig. 4), a periodicity that generally resulted in two pulses of settlers per lunar month. The highest positive autocorrelations after lags of 1-3 days suggested that settlement was not stochastic, but the values were only between 0.2 and 0.4, which might be attributed to a number of causes. First, there was tremendous variation in the density of settlers among the different settlement peaks, which ranged upwards of two orders of magnitude (Fig. 3). Second, while many settlement

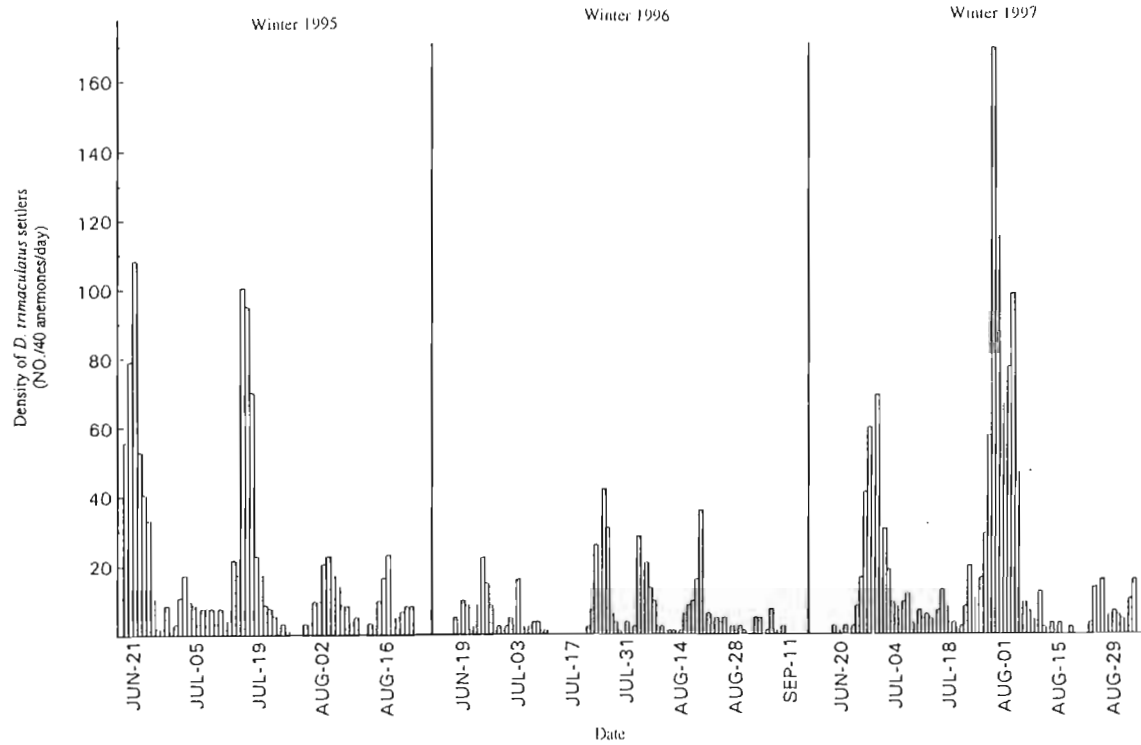


Fig. 3. - Daily pattern of colonization of *Dascyllus trimaculatus* during the winter settlement seasons of 1995-1997. Data are the number of new settlers counted each day on 40 anemones from which residents and settlers were regularly removed.

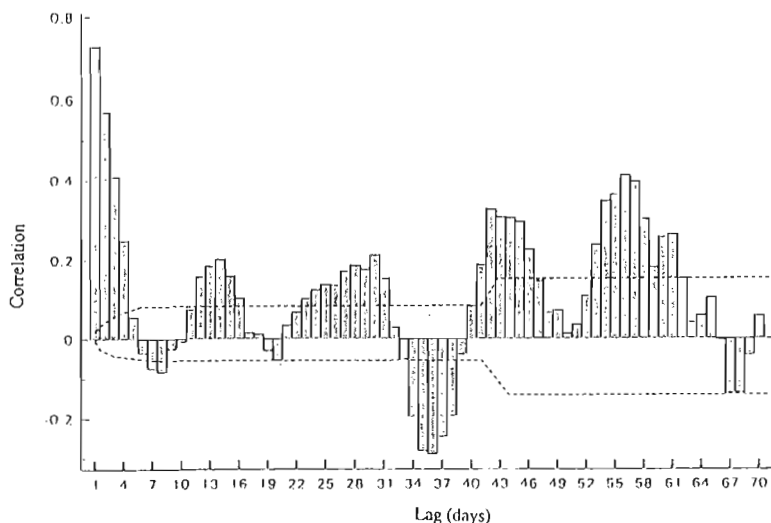


Fig. 4. - Autocorrelations in daily settlement densities of *Dascyllus trimaculatus* as a function of the lag interval. Data are the correlation coefficients for lag intervals of 1 to 70 days, and the dashed line represents two standard errors.

pulses were brief in duration (3-5 days), some pulses occurred over 7-10 days (Fig. 3). Pulses that were longer tended to be those with greater densities of settlers during the peak days of the event (Fig. 3). Third, it was not uncommon for there to be just a single pulse in a lunar month instead of two (Fig. 3). Fourth, the exact timing of settlement in relation to the quarter moon phases of the lunar month was not constant; peak settlement could occur well before, relatively near or well after the actual date of the quarter moon. Finally, several settlement events appeared to be bi-modal, consisting of two distinct closely-timed peaks instead of a single sharp peak (Figs 3, 5). Nonetheless, the temporal pattern of daily settlement of *D. trimaculatus* generally was regular and predictable with a ~14 day periodicity. However, the amplitude of the cycles (i.e., the total density of settlers that arrived during a settlement cycle) was far less predictable.

Our estimates of daily settlement of all three species of *Dascyllus* for 5 different two-week long cycles of settlement revealed relatively high concordance in the timing of peak settlement among the species (Fig. 5). Except in one case where peak settlement of *D. flavicaudus* occurred 4 days earlier than the others (Fig. 5c), maximum settlement of all species was within a day of one another (Fig. 5). One of the five two-week periods was notable for the multiple, concurrent peaks in settlement of all three *Dascyllus* species (Fig. 5d). Rather than the typical single, relatively sharp peak, settlement of each species during this period was characterized by 2-3 peaks separated by 1-2 days of much lower settlement (Fig. 5d). Since all three species showed the same qualitative pattern, the cause likely was related to some unusual external driving force such as the strong south wind that developed during this settlement period but not the others.

Like *Dascyllus trimaculatus* (Fig. 3), the total density of settlers of *D. flavicaudus* and *D. aruanus* that arrived in a pulse varied among the two-week settlement periods by about 1.5 orders of magnitude (Fig. 6). There was high temporal concordance in the strength of settlement events between *D. trimaculatus* and *D. flavicaudus* (Fig. 6): when

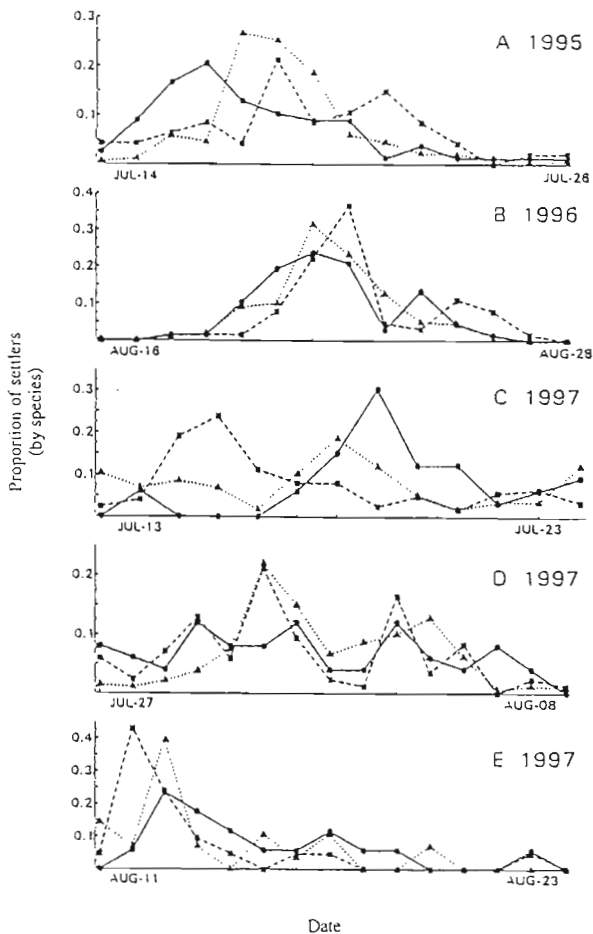


Fig. 5. - Temporal coherence in the timing of settlement of three species of *Dascyllus* at Moorea, French Polynesia, for five different two-week long settlement cycles (labeled A through E, followed by year of observation). Data are the proportion of settlers of a species in a cycle that colonized each day for a given settlement cycle. *D. trimaculatus* is represented by triangles and dotted line, *D. flavicaudus* by squares and dashed line, and *D. aruanus* by circles and solid line.

one of these species settled in large or small numbers, so did the other. However, there was no correlation among settlement events in the density of *D. aruanus* settlers and that of either *D. flavicaudus* or *D. trimaculatus* (Fig. 6).

## DISCUSSION

Damselfishes in the genus *Dascyllus* at Moorea, French Polynesia showed considerable temporal variation in the settlement of larvae from the plankton to the reef environment. All three species examined displayed qualitatively similar temporal patterns, and, for the most part, fluctuations were reasonably predictable with respect to periodicity



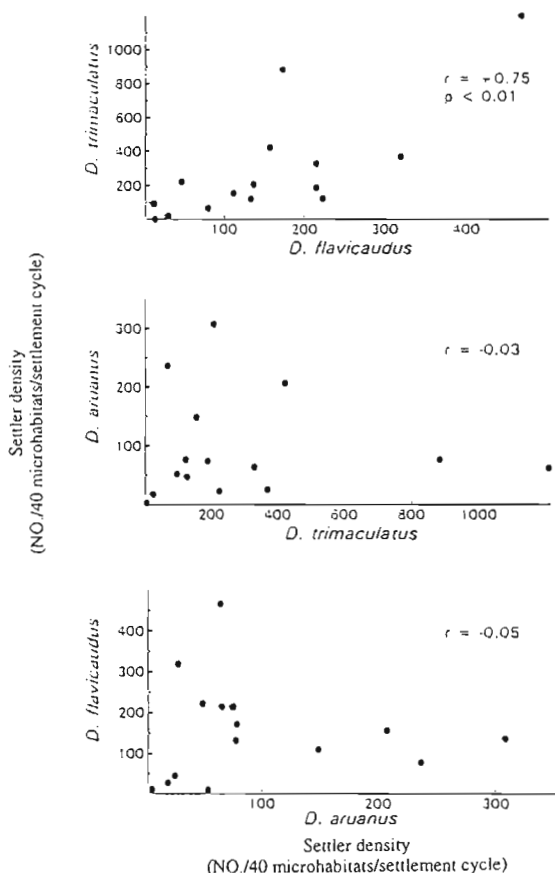


Fig. 6. - Temporal concordance among the *Dascyllus* species in the magnitude of settlement. For each pairwise combination of the damselfish species, the joint densities of settlers (number per 40 microhabitats) that arrived in each of 15 two-week long settlement cycles are plotted together; correlation coefficients also are given.

or timing of settlement events. While *Dascyllus* larvae settled throughout the year, there was strong seasonality with the greatest colonization occurring during Austral winter months. Seasonality in larval settlement of coral reef fishes has been reported for many other locations, including the Great Barrier Reef (Williams and Sale, 1981; Williams, 1983; Williams *et al.*, 1994), Hawaii (Booth, 1992), the Caribbean (Munro *et al.*, 1973; Luckhurst and Luckhurst, 1977; Booth and Beretta, 1994), the eastern Indian Ocean (Doherty and MacIlwain, 1996), southern Japan (Ochi, 1985), Guam (Kami and Ikehara, 1976) and the northern Red Sea (Fishelson *et al.*, 1974). However, even where considerable seasonality has been documented, such as in the Caribbean, some species of reef fishes can show relatively little variation in colonization rates among the seasons (e.g., MacFarland *et al.*, 1985).

Lunar periodicity in settlement of coral reef fishes has been detected for many different species. When a lunar cycle has been found, settlement typically peaks near the new moon period (Sale, 1985; Doherty and Williams, 1988; Robertson, 1992; Dufour *et al.*,

1996; Sponaugle and Cowen, 1997). As settlement to coral reefs appears to occur mainly at night, the typical lunar pattern of settlement during the darkest phase of the lunar month has been suggested to be an evolved response that reduces mortality from visual predators during the period of transition from the plankton to the reef (Sale, 1985; Doherty and Williams, 1988; Doherty, 1990). However, many species of tropical pomacentrids and some labrids with a lunar settlement cycle primarily colonize the reef during one or both quarter moon phases rather than the darker new moon period (Nakazono *et al.*, 1979; Pressley, 1980; Schmale, 1981; Williams, 1983; Robertson *et al.*, 1988; Milicich *et al.*, 1992; Robertson, 1992; Meekan *et al.*, 1993; Milicich and Doherty, 1994; Sponaugle and Cowen, 1996; Sponaugle and Cowen, 1997). The three species of *Dascyllus* we examined showed this pattern of highest settlement near quarter moon periods and considerably lower colonization during both full and new moon phases. Like most other species, settlement peaks for these *Dascyllus* tended to be brief in duration (~3-5 days), although the exact timing of the peaks with respect to quarter moon periods was not constant among settlement events. However, the underlying periodicity of the settlement cycle for these *Dascyllus* was ~14 days, which generally resulted in two pulses of settlement each lunar month.

Two factors have been identified as possible determinants of observed lunar patterns in settlement of reef fishes. One is periodicity in production of eggs of species with relatively fixed durations of the planktonic stage (Robertson *et al.*, 1988). For example, Meekan *et al.* (1993) found that the cyclic nature of settlement of the damselfish *Pomacentrus amboinensis* on the Great Barrier Reef likely was driven by the temporal pattern of spawning. Adult *P. amboinensis* tended to spawn benthic eggs near times of new and full moon, and the timing of subsequent peaks in settlement matched well the incubation period of benthic eggs plus the duration of the planktonic phase (Meekan *et al.*, 1993; also see Milicich and Doherty, 1994). Robertson *et al.* (1988) reached a similar conclusion for lunar periodicity in settlement of the Caribbean damselfish *Stegastes partitus*. In addition, variation in tidal amplitude associated with lunar cycles also has been identified as having a role in shaping temporal patterns of settlement. Sponaugle and Cowen (1997) found that the timing of settlement of many Caribbean labrids was well predicted by the maximum tidal amplitude during a lunar month, and suggested that monthly patterns may be due to variable transport of late-stage larvae to reefs by onshore flood tides. However, since maximum tidal amplitudes typically are associated with new and full moon phases, it is difficult for this mechanism to explain consistent patterns of peak settlement during quarter moon periods, as has been observed for many species of coral reef fishes including the species of *Dascyllus* we studied.

The observation that many species have much lower settlement during the darkest moon phase than the quarter moon periods suggests that the 'avoidance of visual predators while settling' hypothesis is not a complete explanation for the lunar timing of larval colonization. Morgan and Christy (1995) proposed that the mortality of newly-hatched larvae from visual predators would be minimized if they were released at night during the greatest amplitude tides of a lunar month. Tidally-driven current flow is predictably the greatest during spring tides (new and full moon phases), which may enhance the transport of newly-hatched young away from reef environments that contain abundant predators of larvae. Since the larvae of many damselfishes appear to have relatively fixed durations of around three weeks (Brothers *et al.*, 1983; Wellington and Victor, 1989), synchronous spawning during periods of maximum tidal amplitude would result in settlement peaks around the quarter moon phases of the lunar month. When examined, damselfishes have

shown maximum spawning around the new moon with some having a second maximum near the full moon phase (Robertson *et al.*, 1988; Meekan *et al.*, 1993; Milicich and Doherty, 1994). This possibility remains to be explored as a contributor to the temporal patterns of settlement of *Dascyllus* at Moorea, particularly since tidal flux may have less influence than the offshore swell climate on current conditions at this location (see Bongers and Wyrki, 1987).

In addition to the timing of settlement, most species of coral reef fishes examined have displayed considerable variation in the intensity of settlement (i.e., total density of colonists) among different pulses. The *Dascyllus* species were no exception. However, the two most common species (*D. trimaculatus* and *D. flavicaudus*) showed high temporal correlation in the magnitude of settlement among pulses, suggesting that some common processes influenced variation in peak densities of colonists. While temporal pattern in spawning is a plausible mechanism for the general seasonal and lunar cycles of settlement displayed by the *Dascyllus* species, it is less likely that this explains much of the variation in the intensity of settlement among different pulses within a season. Meekan *et al.* (1993) made a similar judgement for the damselfish *Pomacentrus amboinensis*, as did Robertson *et al.* (1988) for *Stegastes partitus*. Reproductive patterns may only have influenced the timing of settlement events rather than their magnitude. Like others (Milicich and Doherty, 1994), these workers presumed that variability in settler densities not explained by reproduction was caused by planktonic processes. A promising area of future inquiry is to relate variation in production and subsequent fate of early life stages with variation in physical transport processes (e.g., Gaines and Bertness, 1992).

**Acknowledgements.** - We thank Keith Seydel, Craig Shuman, Steve Larsen, and Pete Raimondi for assistance in the field, Bonnie Williamson, Kristen Zabaronick, Will Holbrook and Melissa Schmitt for logistical assistance, two reviewers for comments and René Galzin for assistance with this manuscript and in organizing the symposium at the 5th Indo-Pacific Fish Conference where the paper was presented. We appreciate the hospitality of Frank Murphy and Steve Strand of the UC Berkeley Gump South Pacific Biological Station. The research was supported by the National Science Foundation (OCE 95-03305) and funds for instrumentation were provided by the W.M. Keck Foundation. This is Contribution No. 60 of the Gump South Pacific Biological Station.

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## **AN EXAMINATION OF THE EFFECT OF ECOLOGICAL FACTORS, ESPECIALLY COMPETITIVE EXCLUSION, ON THE DISTRIBUTIONS OF SPECIES OF AN INSHORE, TROPICAL, MARINE FAMILY OF INDO-PACIFIC FISHES (SIGANIDAE)**

by

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**ABSTRACT.** - The distribution patterns of species and subspecies of any family of organisms have been determined by three kinds of factors: historical episodes, dispersal abilities and niche requirements. In other words, distribution patterns can be interpreted in both an evolutionary and ecological context. Recognition of the possible consequences of plate tectonics and changes in sea level associated with glaciation episodes has led to an emphasis on evolutionary factors in the recent literature. However, ecological factors also play a role. Intolerance to low temperatures clearly plays a decisive role in influencing the latitudinal range of tropical fishes, and the availability of a particular type of habitat can clearly also determine the ranges of species. A detailed analysis of the distribution of sibling taxa of Siganidae indicates that competitive exclusion plays a significant role in limiting the distributions of more than half the species of the family.

**RÉSUMÉ.** - Analyse de l'influence des facteurs écologiques, notamment de l'exclusion par compétition, sur les distributions des espèces d'une famille de poissons tropicaux littoraux de l'Indo-Pacifique, les Siganidae.

La distribution des espèces et des sous-espèces de n'importe quelle famille d'organismes est habituellement déterminée par trois types de facteurs: les épisodes historiques, la capacité de dispersion des espèces et les exigences du milieu. Autrement dit, la distribution des espèces peut s'interpréter dans un contexte évolutionniste et écologique. La reconnaissance des conséquences possibles du mouvement des plaques tectoniques et des changements du niveau de la mer associés aux épisodes glaciaires a récemment engendré une abondante littérature sur ces facteurs évolutionnistes. Pourtant, les facteurs écologiques jouent aussi un rôle. En particulier, la non tolérance des basses températures intervient de manière décisive en influençant la répartition en latitude des poissons tropicaux et, de la même façon, l'existence d'un type particulier d'habitat peut aussi déterminer la répartition des espèces. Une analyse détaillée de la distribution d'espèces sœurs de Siganidae indique que l'exclusion par compétition joue un rôle significatif en limitant les distributions de plus de la moitié des espèces de cette famille.

**Key-words.** - Siganidae, ISEW, ISW, Indo-Pacific, Competitive exclusion, Zoogeography, Geographic complementarity.

The distribution patterns of the members, e.g., species and subspecies, of any family of organisms have been determined by three kinds of factors: historical episodes, dispersal abilities and niche requirements. In other words, distribution patterns can be interpreted in both an evolutionary and ecological context. Nevertheless, theories about

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the zoogeography of Indo-Pacific fishes often involve only historical aspects such as plate tectonics (Springer, 1982) or Pleistocene sea level changes associated with alternating glacial and interglacial periods (Woodland, 1986). Ecological aspects are often ignored; yet Ecology is the study of how physical and biotic factors determine the distributions and abundances of organisms.

There is, of course, ample evidence that a whole range of environmental factors can affect population densities. More importantly, in the present context, however, both physical factors such as temperature, salinity, turbidity, and bottom type, and biological factors such as interspecific competition are known to be able to influence the distributions of marine species (Connell, 1961; Tobias, 1976; Race, 1982). Not all of these components can be considered in depth for the Siganidae; instead the effects of temperature and habitat type will be looked at cursorily, while the more controversial role of interspecific competition will be looked at in more depth.

### The Siganidae

Woodland (1990) recognised 27 species of Siganidae; but another one is to be described, while two previously considered as subspecies need to be elevated to the rank of species, making a total of 30 species. Certain characteristics of the species are relevant to what is discussed later, especially their habitats, resources, distributions and ability to disperse. The following summary is based on a comprehensive literature review and many hundreds of hours collectively of underwater observation of almost all of the species by the author.

Rabbitfishes seldom grow larger than 40 cm. They are primarily herbivorous, feeding mostly on bottom algae, though a pair of sibling species also feeds on sponges and colonial tunicates. The larvae feed on both zoo- and phytoplankton (May *et al.*, 1974; Popper *et al.*, 1979). Half the species are found only on coral reefs and 13 of these live in pairs among the branching corals; all other species live in schools. Most of the species that are not restricted to coral reefs (for example, they may also feed on algae growing on rock reefs) are, nevertheless, common about coral reefs. The precise diet of most species is unknown. They take any small flesh bait but feed selectively on algae; e.g., adults of *Siganus fuscescens* (as *S. oramin*) fed on only 11 of the 101 species of algae present in their habitat (von Westernhagen, 1973).

Fishermen report that *Siganus guttatus* is unusual in that it is active at night, while all the pair-forming coral dwelling species and probably all of the schooling species found on reefs sleep at night. Those fish visible to a diver sleep up against a vertical face of the dead base of a coral clump or under the lip of beachrock or in sea grass. Sleeping places provide protection against night predators. Whether safe sleeping places may be a limiting resource in some habitats is not known.

Through extensive collecting by himself and colleagues and an examination of all the significant institutional collections, the author was able to map accurately the distributions of each species and subspecies of siganid (Woodland, 1990). Further study since the publication of the revision suggests that cryptic species may need to be dissected out of *Siganus fuscescens*, but any taxonomic changes will not affect the conclusions reached here.

Little has been published on the biology of siganid larvae; there is nothing on the pairing species, but the details are well known for some of the schooling species considered suitable for aquaculture, e.g., *S. vermiculatus* (Gunderman *et al.*, 1983); *S. fuscescens* (May *et al.*, 1974). In all species investigated, larval life span was three to four weeks and



larvae actively maintained themselves near the water surface. These features are conducive to larvae being transported by sea surface currents. Major sea surface currents in the Indo-Malayan/Western Pacific area such as the Pacific North and South Equatorial Currents and Equatorial Countercurrent, the East Australia Current, Kuro Current, Leeuwin Current, and other lesser currents in the Celebes, Java and Banda Seas have speeds of 1-1.5 km/hr. A conservative estimate of the potential distance larvae could travel passively within these currents is 500 to 1,000 km.

### Physical factors affecting distributions

The Siganidae is a tropical fish family with species richness declining towards higher latitudes: e.g., 12 species co-occur in a relatively small area like the Belau Islands (7°30'N), ten at the northern end of the Great Barrier Reef, eight at the southern end, three at the latitude of Brisbane, but only one on the central coast of New South Wales. The figures are similar as one progresses towards higher latitudes in the northern hemisphere: nine species for the Ryukyu Islands, but only two for southern Honshu. There is a similar attenuation on the east coast of Africa from four species at the equator to only a single species below 30°S (Woodland, 1990, Fig. 1).

It seems almost trite to point out that this attenuation in the number of species with increasing latitude must be due, at least in part, to lower sea temperatures at higher latitudes. The role of temperature is confounded, however, by the fact that a very sharp decline in species number occurs north and south of, respectively, where the coral reefs of the Ryukyu Islands and the Great Barrier Reef terminate. Since the high latitude limits for reef building corals are themselves temperature determined, it is impossible to say - apart from the disappearance of the obligate corallophilic species such as *S. doliatus* and *S. vulpinus* - whether the loss of fish species is due directly to loss of habitat or to inhospitable temperatures. Hobbyists employ temperatures around 25°C to maintain coral-reef associating species in aquaria. A specimen of the foxface *Siganus (Lo) unimaculatus* - an obligate corallophile kept by the author in a tank at 27°C became lethargic and failed to eat over several weeks, with a considerable loss in condition, when a faulty heater was not detected and water temperatures fell to the mean ambient air temperature of about 18°C.

The siganid with the greatest latitudinal range and therefore presumably the greatest tolerance to low temperatures is *Siganus fuscescens* (Woodland, 1990). Its latitudinal range extends to 30°N and 30°S (on the coasts of Japan and New South Wales) even in the cooler months; but as the prevailing warm currents in both these areas push further north and south, respectively, with the onset of summer, which is also the spawning season of the species, juveniles are found at even higher latitudes. In Japan, this species occurs on the central coasts of Honshu only in autumn (I. and R. Nakamura, pers. comm.). Matsuura *et al.* (1988) found it, along with around a hundred other species usually found only in southern Japan, in autumn on the northern tip of Honshu (41°N), in an area directly exposed to the warm Tsugaru current. On the coast of New South Wales, Australia, juveniles have been found as far south as 37°26'S. A survey on this coast at Jervis Bay (35°S) showed that juveniles of this and other tropical species appeared there in summer. As sea temperatures began to fall the fish became lethargic; divers found them easy to catch with hand nets. All had disappeared by winter, no doubt consumed by predators (J. Paxton, pers. comm.).

A siganid whose range is definitely restricted by its habitat requirements is *Siganus vermiculatus*. It is an estuary-dependent species which is common throughout the Indo-Malayan region. The larvae are planktonic and the fry occur up to at least 25 km out

to sea; the fry migrate into estuaries and coastal lagoons (Gunderman *et al.*, 1983). The juveniles and adults are found either in these habitats or on silted up rock reefs affected by river discharge. In the Western Pacific there is an abundance of high islands with large rivers below the equator, but few such habitats occur in the mainly low, coralline islands of Micronesia north of the equator. This species occurs as far west as Fiji in the southern hemisphere, but in Micronesia it is known from only Guam and Belau, the island groups nearest to the central Indo-Malayan area. Even in the Belau Islands, a mixture of high islands of volcanic origin and low islands of coral reef origin, this species was reported by local fishermen to be found only at the mouth of the single large river that occurs on Babelthaup I. This population survival must be precarious; a prolonged drought or, in the longer term, the loss of the estuary due to a fall in sea level would lead to its extinction. Other species whose distributions would appear to be restricted because of the lack of suitable habitats in eastern Micronesia and Polynesia, namely mangrove flats and seagrass beds (McCoy and Heck, 1976; Woodroffe and Grindrod, 1991), are *S. canaliculatus*, *S. fuscescens* and *S. lineatus*.

Randall *et al.* (1997) surveyed the fishes of the high latitude Ogasawara Islands and found that several species reported as "conspicuous" in previous surveys were not seen by them during their study, and vice versa. Although species of tropical origin made up a third of the fauna, many were present at very low densities - sometimes just a single representative was seen. They speculated that since these islands are in the path of both the cool Kuroshio Countercurrent and the warm Subtropical Countercurrent, that variability in the relative influence of these currents in the area may create a dynamic situation. In some years, larvae of tropical species might be carried in the Subtropical Countercurrent to the islands, where water temperatures might be high enough for the fish to survive and grow but not reproduce.

Marine organisms of tropical origin appear annually on temperate coasts of southwestern Australia (Maxwell and Creswell, 1981). These organisms must be transported to the area by the Leeuwin Current, an intrusion of tropical water that flows southward along the continental slope of western Australia (Legeckis and Creswell, 1981). For example, each year young recruits of up to 20 tropical species of fishes appear at the offshore island of Rottnest (32°S). Their appearance in March or April of most years coincides with the time when the Leeuwin current strengthens and intrudes into the area (Hutchins and Pearce, 1994). These researchers concluded that most of the fish were being recruited from the islands of Houtman Abrolhos (29°S) 300 km to the north, though some species most probably originated from the coral reefs of Ningaloo (22-24°S), a further 450 km north.

Perhaps the most significant message in this section is that siganid larvae, in particular, and marine shorefish larvae in general, are capable of being dispersed by currents over considerable distances to localities well outside their usual range. If not consistently, then occasionally, larvae must arrive in sufficient numbers to be able to establish viable populations in these localities, but they are often thwarted by physical and, as we shall now see, by biotic factors in the alien habitats.

#### **Biotic factors affecting distributions**

The Siganidae contains seven groups of sibling species consisting of pairs or triplets, and all five species in the subgenus *Lo* (Woodland, 1990). Almost all of the sibling taxa within each group exist parapatrically (i.e., their ranges are essentially non-overlapping but have a shared border) (Table I). [The few exceptions are species such as *S.*

(*Lo uspi* in Fiji and *S. (Lo) niger* in Tonga; even here it could be argued that these species exist parapatrically, since no suitable habitat, only open sea, separates the localities.] This pattern is not unique; Allen (1972) recorded that there were four pairs of sibling species with the same attribute within the genus *Amphiprion*. He proposed that these "geminate pairs" may have evolved allopatrically, the isolating mechanism being emergent land barriers during the late Pliocene and Pleistocene glacial periods. He then asked why, if this were so, the distributions of the geminate pairs of *Amphiprion* did not now overlap, the geographic isolating mechanisms having disappeared with the rise of sea levels to their current levels. The most likely explanation in the opinion of the present author is that these species maintain their present distributions by "competitive exclusion".

The "competitive exclusion principle" asserts that complete competitors cannot coexist indefinitely (Hardin, 1960). This principle is supported for two competing species by the classic Lotka-Volterra models. The models' predictions have been confirmed experimentally in the laboratory and in the field (Hassell, 1976). That two species which are complete competitors may coexist under special circumstances, namely when each species inhibits its own population growth more than that of the other species does not affect the line of reasoning which follows.

Consider, then, an ancestral species which has become divided into two entities which have evolved in geographical isolation to become separate species. Should these two entities come into contact again, by removal of the barrier, an alteration to current patterns, or whatever, the daughter species could already be sufficiently different in resource use, for example diet, for each to be able to coexist in sympatry. Alternatively, the daughter species may depend upon the same limiting resource for their survival. In this case, some post-contact evolutionary adjustment in resource use (i.e., "character displacement") would need to occur before the daughter species could coexist sympatrically; failing that, each species would exclude the other from areas of potential overlap, i.e., competitive exclusion.

While it is possible that one or other of the competing species would be driven to total extinction, a likely outcome is that one species would gain the ascendancy in the habitat to which it was best adapted, and vice versa. The end result would be the daughter species occupying exclusive, or virtually exclusive, but contiguous ranges. Such parapa-

Table I. - The pairs and triplets of sibling taxa of *Siganus* that have parapatric, i.e., contiguous, allopatric distributions. Maps showing the distributions of the sibling groupings occur in Woodland (1990), and in the case of the undescribed species on the Western Australian coast [n.sp. (W.A.)] in figure 1 in this paper.

Groups of sibling taxa of <i>Siganus</i> with parapatric distributions		
<i>S. corallinus</i>	<i>S. trispilos</i>	n. sp. (W.A.)
<i>S. doliatus</i>	<i>S. virgatus</i>	
<i>S. puellus</i>	<i>S. puelloides</i>	
<i>S. spinus</i>	<i>S. luridus</i>	
<i>S. lineatus</i>	<i>S. guttatus</i>	
<i>S. punctatus</i>	<i>S. stellatus stellatus</i>	<i>S. stellatus laqueus</i>
<i>S. vulpinus</i>	<i>S. unimaculatus</i>	<i>S. magnificus</i>

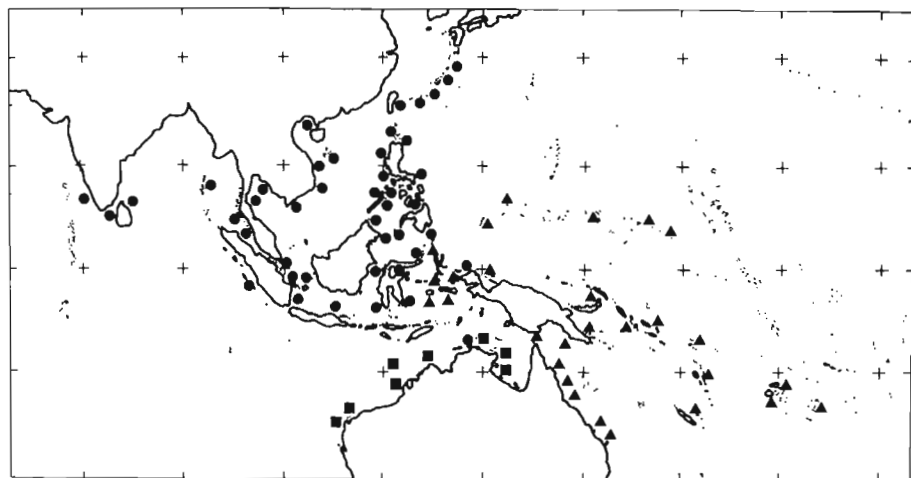


Fig. 1. - Distributions of *Siganus virgatus* (circles), *S. doliatus* (triangles), and an undescribed species of *Siganus* (squares). The distributions of these sibling species are virtually non-overlapping but contiguous.

tritic distributions are displayed by seven sibling species groups within the family Siganidae. An example of these kinds of distributions for one sibling group (*S. doliatus*, *S. virgatus*, and an undescribed species previously regarded by Woodland (1990) as only a colour variant of *S. doliatus*) is shown in figure 1. Maps of the distributions of other sibling groups, all of which show the same kinds of exclusive ranges of their members, can be found in Woodland (1990): *S. corallinus* and *S. trispilos* (fig. 8); *S. spinus* and *S. luridus* (fig. 9); *S. puellus* and *S. puelloides* (fig. 13); *S. punctatus*, *S. stellatus* and *S. laqueus* (fig. 16); *S. lineatus* and *S. guttatus* (fig. 19); and all five species of foxface, subgenus *Lo* (fig. 22). That is, a total of 19 of the 31 recognized distinct taxa of Siganidae apparently have their distributions restricted by congeners. All sibling groups show this pattern with the exception of *S. fuscescens* and *S. canaliculatus* whose ranges overlap considerably (Woodland, 1990, fig. 12); in this case the species prefer different habitats, reefs and non-reef areas, respectively.

## DISCUSSION

One could raise various objections to this interpretation of the observed distributions. First, why do the sibling species occupy clearly definable, geographic areas from which the other species is totally excluded (except along the common border, presumably) rather than their distributions being mixed in some mosaic pattern? In other words, it seems unlikely, though not impossible, that an area exclusive to one sibling would be so uniform in its physical and biological attributes that the excluded sibling was unable to find pockets where it was competitively superior.

The likely answer is that such pockets do exist, but that emigration by the victorious sibling from surrounding areas by individuals surplus to replacement excludes the other sibling from areas where it might maintain viable populations in the absence of this emigration. In the Lotka-Volterra competition model (excluding the situations which lead

to a stable or unstable unequilibrium between the two species populations), the victor is the stronger interspecific competitor of the two (Hassell, 1976). Assuming similar intrinsic rates of increase and similar potential carrying capacities for each species in a contested area, it is the species which achieves the highest rate of increase that will be the victor. The rate of increase is treated as though it were due entirely to reproduction. However, in a field situation, reproduction may be supplemented by immigration. Therefore, it is the species which has the highest mean rate of increase, averaged over all areas being contested, which will dominate an area. In this context, larvae of a particular species carried by a current into an area that might have supported either of two species may determine which species establishes itself in that area. This might explain, for example, why the siganid fauna of the Ryukyu Islands more closely resembles that of the Indo-Malayan area rather than Micronesia, larvae being transported in the Kuro current north to the archipelago.

An objection to the thesis of competitive exclusion affecting distribution will be raised by those researchers who believe food appears not to be a limiting resource for coral reef fishes (Goldman and Talbot, 1976). However, as Ebeling and Hixon (1991, p. 544) have noted, some "recent experimental and circumstantial evidence suggest otherwise". Indeed, they include "geographic complementarity" between related species, the kind of distributions reported here, as circumstantial evidence for competition. Be that as it may, direct observations of the pairing species on coral reefs by the author reveals very frequent interference between the same species and between different species of siganid, i.e., threat posturing and chasing of one pair by another both within and between species, e.g., between *S. doliatus*, *S. vulpinus* and *S. corallinus* at the one site (unpubl. data). Such "interference" provides only circumstantial evidence for competition. Competitive exclusion occurs when two species are faced with an insufficient supply of a single limiting resource (Hardin, 1960); e.g., a single species of alga. Siganids have multispecies diets, so it could be argued that since it is unlikely that two sibling species would rely on the same limiting resource that competitive exclusion would occur. But it should be pointed out that the Lotka-Volterra models of interspecific competition do not include a resource parameter. They can be interpreted as "interference" rather than exploitation models. In other words, "interference exclusion" rather than "competitive exclusion" could occur even though resources were in an absolute sense not limiting.

It is worth noting that the distributions of the three sibling species of siganid in figure 1 share a number of features in common with the distributions of three sibling species of *Amphiprion* depicted in Fautin and Allen (1992). *A. frenatus* matches *S. virgatus* except that *A. frenatus* does not range into the northern Indian Ocean; *A. melanopus* matches *S. doliatus* except that *A. melanopus* ranges further eastward than Tonga to south-eastern Polynesia; and *A. rubrocinctus* has an identical distribution to the undescribed sibling species of *Siganus*. That there should be coincidence between the combined distributions of both sets of siblings is not surprising; both groups live on coral reefs, the anemonefishes because their host lives almost exclusively there. The remarkable feature is that the shared borders between both sets of siblings are essentially identical. This is curious because the limiting resource for the three *Amphiprion* species may well be the availability of their anemone host, *Entacmaea quadricolor*. Although the host is used by other species of *Amphiprion* whose distributions coincide with the siblings, only rarely do any of the siblings use other host species. There is thus considerable potential for competition for hosts; but why should the common boundaries between the siblings of the two groups be so similar when the resources for which they might compete are so

different? Perhaps the answer lies in differences in qualities of the different water bodies in the boundary area: the Celebes/Java Seas of the Asian continental shelf, on the one hand, and the off-shelf Banda and Timor Seas, on the other. For example, unlike the deep waters surrounding the islands of Banda and Timor Seas, the Java Sea is shallow, mostly less than 50 m, and the salinity is normally low due to the high rainfall on the surrounding lands; in general, the bottom is muddy, the turbidity too high and salinity too low to support luxurious coral reefs (Tjia, 1966). Differences in the structure and species composition of the benthic communities in the two areas may favour one species in one area and its sibling in the other.

Determining how one species might gain an advantage over another could be extremely difficult. Probably the best known "experiment" on competitive exclusion was where the introduced hymenopteran parasite, *Aphytis chrysomphali*, of the California red scale (*Aonidiella aurantii*) was fully displaced from the citrus-growing areas of southern California by a later introduction, *A. linganensis*, which was itself later displaced over much of its range by a further introduction, *A. melinus* (DeBach and Sundby, 1963). Despite the fact that the events were monitored as part of a biological control program, that the systems involved were very simple, and that many laboratory experiments were conducted, the exact mechanisms by which exclusions occurred remained elusive. Luck and Podoler (1985) concluded that the decisive factor that gave *A. melinus* its competitive edge over *A. linganensis* over most of the contested area was that *A. melinus* accepted a smaller threshold size of scale for the production of daughters.

Competitive exclusion of one species by another is impossible to prove once the process is complete; although complete or partial removal of one of the competitors in order to allow the process to be reversed might be used to obtain supporting evidence. As DeBach and Sundby (1963) observed, they were fortunate to be studying the very populations of *Aphytis* spp. at the time during which competitive exclusion was occurring. They were involved in a controlled experiment involving the introduction of alien species. Such "experiments" are being repeated on a large scale today by the intentional or unintentional introduction of organisms into areas where they previously did not exist, often with disastrous consequences for the native species, e.g., the introduction of marine organisms in bilgewater. The exact mechanisms by which alien species suppress native species may be difficult to establish, nevertheless competitive exclusion is often suggested. For example, various species of *Gambusia* have been introduced into several countries for the control of mosquitoes. These introductions have impacted on at least 35 native fish species, causing a decline in their distribution or abundance (Schoenherr, 1981; Lloyd, 1990). The mechanism of displacement by *Gambusia* is complicated by the fact that they may prey on the eggs or fry of fishes, but both exploitation and interference competition play a role (Lloyd, 1990). The *Gambusia* harass other fishes by chasing and fin nipping. Similar behaviour has been observed by the author between *Siganus* spp. In adults of pairing species it appears to be more intense between members of the same species than other congeners. Data are needed on the responses between sibling species where the species co-occur.

Returning to Allen's (1972) thesis that the isolating mechanisms for the evolution of these sibling taxa may have been created during the times of sea-level low over the last 500,000 years or so, we need to obtain estimates of times of divergence from for example mtDNA to see if this is plausible. Even then, we will still be forced to speculate about where the isolates were located as they diverged. For example, did *S. virgatus* diverge while isolated in the semi-enclosed basin which is now the South China Sea or was

the isolate in Southern India? One thing we are sure of is that during the time of maximum sea level recession of 150 metres or more only 20,000 years ago, *S. virgatus* could not have occurred over much of the area of S.E. Asia which it occupies today; that area was dry land (Potts, 1983). Similarly the exposure of the Northwest Shelf of Western Australia and the joining of northern Australia to southern New Guinea would have deprived the undescribed sibling of most of the territory it occupies now. Even the Great Barrier Reef did not exist. With the return to present day sea levels, the distributions we observe today of these species were determined by the availability of suitable habitat. Marginal areas would be "defended" from incursions by species making demands on the same limiting resources. As competition will be most complete amongst the species most closely related to one another, then according to the thesis presented here the results of competitive exclusion will be displayed as non-overlapping distributions of sibling species.

**Acknowledgements.** - The author would like to thank the organisers of the 5th Indo-Pacific Fish Conference for the opportunity to publish this paper and the two anonymous referees for their helpful comments on the manuscript.

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## Symposium 14

### FISH LARVAE

Chairperson: **Jeff LEIS**

- CASTLE P.H.J. & D.G. SMITH. - Precocious ovarian development in leptocephali of three Indo-West Pacific species of ilyophine eels (Teleostei: Synaphobranchidae). 565-574

*Développement ovarien précoce chez les larves leptocéphales de trois espèces d'Ilyophinae (Teleostei: Synaphobranchidae).*

- LEIS J.M. & I.C. STOBUTZKI. - Swimming performance of late pelagic larvae of coral-reef fishes: *In situ* and laboratory-based measurements. 575-583

*Performances de nage des larves pélagiques âgées de poissons récifaux: mesures in situ et en laboratoire.*

- NAKAZONO A. - A method to take photographs of live late larval and juvenile reef fishes. 585-588

*Une méthode pour photographier les stades larvaires avancés et les juvéniles de poissons récifaux vivants.*

- RIEHL R. - Minireview. The micropyle of teleost fish eggs: Morphological and functional aspects. 589-599

*Brève revue. Le micropyle des oeufs des poissons téléostéens: aspects morphologiques et fonctionnels.*



## PRECOCIOUS OVARIAN DEVELOPMENT IN LEPTOCEPHALI OF THREE INDO-WEST PACIFIC SPECIES OF ILYOPHINE EELS (TELEOSTEI: SYNAPHOBANCHIDAE)

by

Peter H.J. CASTLE (1) & David G. SMITH (2)

**ABSTRACT.** - Eels of the subfamily Ilyophinae are generally distinct from those of the subfamily Synphobranchinae in having few but prominent vomerine teeth, anus far forward, separate gill openings and body naked. Adults live near the bottom as deep as 2,000 m while leptocephali live at about 200 m. Larvae of both subfamilies have a distinctively "tubular" eye, but are otherwise different, especially in the presence of a bird-like or extended snout in the Ilyophinae. The 200 study larvae from the Dana and ORSTOM collections in the Indo-west Pacific tentatively comprise about 25 species. Thirty four specimens, of three species, have identifiable ovaries with minute ova. These species are thus possibly paedogenetic, except that the small size of the ova suggests that maturity is unlikely to be attained in leptocephali.

**RÉSUMÉ.** - Développement ovarien précoce chez les larves leptocéphales de trois espèces d'Ilyophinae (Teleostei: Synphobranchidae).

Les espèces de la sous-famille des Ilyophinae se distinguent généralement de celles de la sous-famille des Synphobranchinae par un nombre réduit de dents vomériennes saillantes, un anus situé plus antérieurement, des ouïes séparées et un corps nu. Les adultes vivent sur le fond jusqu'à 2 000 m de profondeur tandis que leurs larves leptocéphales vivent vers 200 m de profondeur. Les larves des deux sous-familles ont des yeux tubulaires, mais elles diffèrent nettement par d'autres caractères tel le museau des Ilyophinae allongé comme le bec d'un oiseau. Les 200 larves étudiées, provenant des collections Dana et ORSTOM de l'Indo-Ouest Pacifique, représentent environ 25 espèces. Trente-quatre larves de trois espèces possèdent des ovaires distincts avec des ovules minuscules. Il est possible que ces espèces soient paédogénétiques, bien que la petite taille des ovules suggère que la maturité est peu probable au stade leptocéphale.

**Key-words.** - Synphobranchidae, Ilyophinae, ISEW, ISW, Indo-West Pacific, Leptocephali, Ovaries, Paedogenesis.

The eel family Synphobranchidae is known from the outer shelf to much greater depths in all oceans. Robins and Robins (1989) reviewed the family in the Atlantic comprehensively but referred only briefly to the Indo-west Pacific species. These authors recognised three subfamilies: Simenchelyinae (1 genus and 1 species), Synphobranchinae (2 genera and 8 species), and Ilyophinae (6 genera and 17 species, including 1 genus and 2 species *added since*). The Simenchelyinae, with its single cosmopolitan species *Simenchelys parasitica* Gill, 1879 is distinct from the other two subfamilies in its blunt

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snout and small, terminal mouth. The boundary between the Synphobranchinae and the Ilyophinae is less well defined and largely concerns the presence or absence of scales, length of lower jaw, nature of gill slits and their position, position of anus, and dentition (Robins and Robins, 1989).

Ilyophines are known from at least four genera and 13 species in the Indo-west Pacific in depths of 100 m (*Dysomma anguillare* Barnard, 1923) to an estimated 2,000 m (*Ilyophis blachei* Saldanha & Merrett, 1982). However, these and other records including several yet to be reported (Castle, unpubl. data) are based on rather few specimens from widely disjunct localities from Tahiti to South Africa. Several of the species are known from just a few specimens each. Inadequate collection in the depths in which the species occur may explain this apparently disjunct distribution.

These fragmentary records may explain why so little is known of the biology of any of the ilyophine eels, except perhaps *Ilyophis brunneus* in the western North Atlantic where it is apparently quite abundant (Martin, 1975; Robins and Robins, 1989). Even less is known of their early life history except that they have a leptocephalous phase, like all other eels. The remarkable leptocephali of the Ilyophinae were, in fact, first described by Schmidt (1909: *Leptocephalus rostratus*; 1913: *L. telescopicus*) and Lea (1913: *L. dolichorhynchus* and *L. proboscideus*) from the North Atlantic, the names referring to the distinctively elongate snout appendage in these species. D'Ancona (1928) described a further species from the Red Sea (*L. synphobranchoides*). However, none of these authors appear to have recognised that larvae of this type were ilyophines. Smith (1974) eventually referred them to the Dysommataidae (now Ilyophinae) though without definitive species identifications.

Synphobranchine and ilyophine leptocephali share "tubular" (or so-called "telescopic") eyes. The eyeball is elongated and directed obliquely upwards, forwards and outwards, the lens is superior and the retina deep, its structure presumably being an adaptation to life deeper in the water column. However, larvae of the two subfamilies are profoundly different in body shape and pigmentation. Synphobranchines are relatively shallow-bodied, the snout is short like that of most other leptocephali, there is a distinctive, pinkish (on preservation) internal band of tissue along the vertebral column, and the body lacks all but pigment on the caudal fin. In contrast, ilyophines are relatively deep-bodied and leaf-like (Fig. 1 A-F) with several gut thickenings, the snout is relatively long, depressed and rather beak-like, and in some it is greatly extended as a whip-like filament or spear-like rostrum. All have distinctive pigmentary patterns to a greater or lesser degree (Castle, 1984).

We have assembled, and have studied in a preliminary way, about 200 ilyophine leptocephali from the Indo-west Pacific, mainly from the Dana Oceanographical Collections, Copenhagen, and ORSTOM, Nouméa. Regrettably, we have made little progress in identifying the various forms and are not confident in doing so. Nevertheless, we have concluded that the collection contains possibly as many as nine genera (based on body shape and pigmentation) and about 25 species (based on numbers of myomeres), i.e., more than are known as adults in the Indo-west Pacific. Synphobranchid leptocephali as a whole are very difficult to identify because metamorphic specimens that usually provide the clues to generic identification are rare even in the extensive collections of leptocephali that we have variously studied. Additionally, in the few juvenile ilyophines that we have seen, the defining vomerine tooth pattern has not yet fully developed. In any case the metamorphic forms almost certainly live near the bottom in moderately deep to very deep water and by their very nature are not readily accessible to capture.

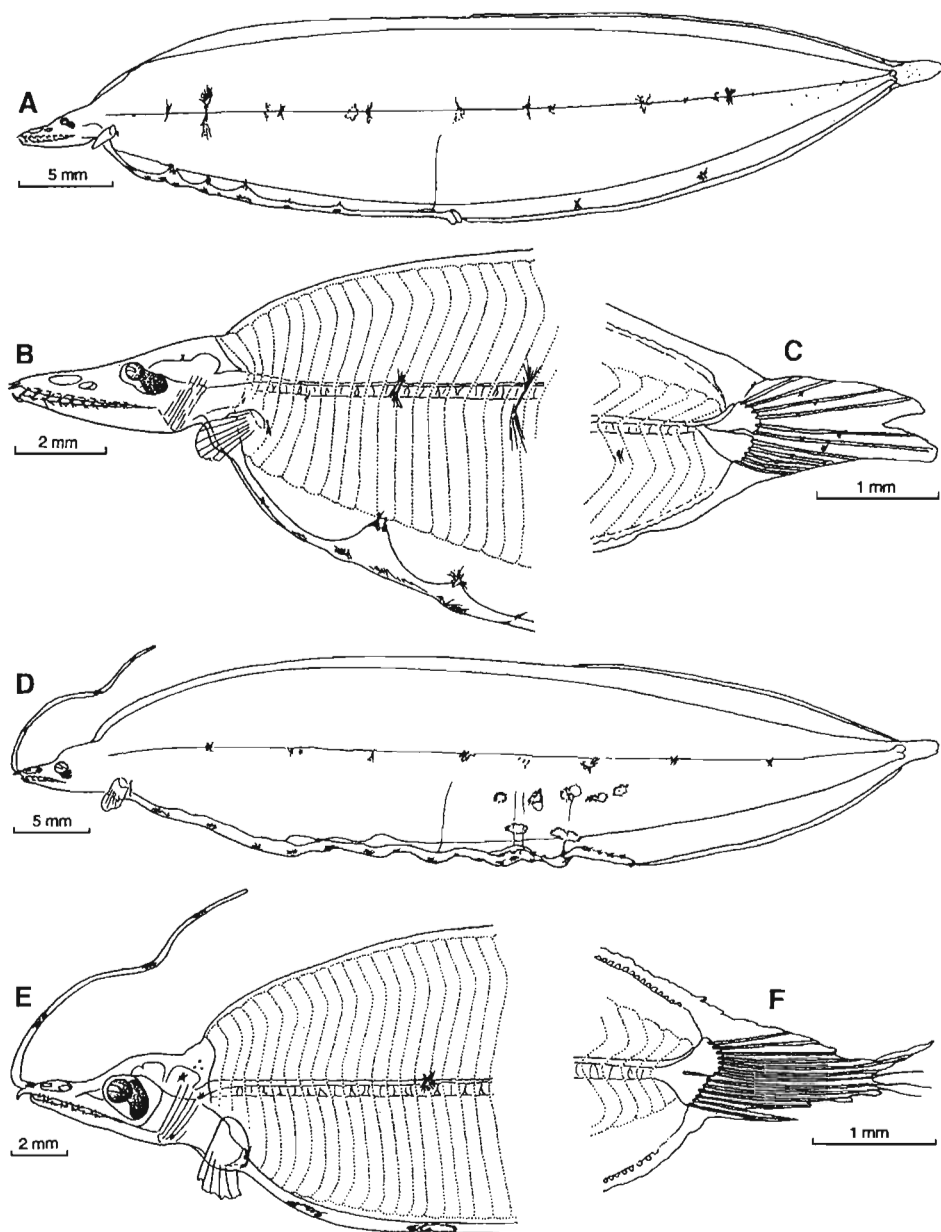


Fig. 1. - A-C: Species B<sub>10</sub>, Dana station 3731, haul VI, 46.0 mm TL, ZMUC P32995, voucher specimen. D-F: Species D<sub>1</sub>, Dana station 3685, haul II, 59.5 mm TL, ZMUC P32996, voucher specimen.

Remarkably, some 34 of the assembled 200 specimens, belonging to three species, possess developing ovaries containing minute ova. Precocious reproductive development in various Anguilliformes and Saccopharyngiformes has already been reported (Castle, 1978), though only in metamorphic specimens rather than in unmetamorphosed

leptocephali. We are not aware of any similar situation in larval fishes. We therefore consider that this extraordinary observation warrants reporting and hence is the subject of this paper.

## MATERIALS AND METHODS

The leptocephali treated in this paper were collected principally by the Danish Dana Expedition Round the World 1928-30 and are held in the collections of the Zoological Museum, University of Copenhagen (ZMUC). Other specimens were collected by ORSTOM, Nouméa during its oceanographic studies in the central and southwestern Pacific 1965-72 and will eventually be deposited in the Muséum national d'histoire naturelle, Paris (MNHN).

In the absence of definitive generic and specific identifications we have adopted the terminology introduced by Smith (1989). This distinguishes between larvae of different body shapes, pigmentation and myomere numbers. Accordingly, those in the categories A and B were termed "non-rostrate" forms (i.e., without a snout appendage and with or without lateral body pigmentation, respectively) and those in categories C and D are "rostrate" forms (i.e., with the snout extended as a filament or spear, also with or without lateral pigmentation). The various groups of species within these categories were assigned numerical subscripts according to range in number of their myomeres.

## CHARACTERS AND REPRODUCTIVE STATES OF THE ILYOPHINE LEPTOCEPHALI

### Subfamily Ilyophinae

*Diagnosis.* - As summarised from Smith (1989): maximum size usually about 60 mm TL, sometimes larger, up to 200 mm TL. Body relatively deep, anus half-way to two-thirds along body; pectoral present; caudal well developed with broad hypurals, each bearing 6-8 rays; gut thickened at several points along length. Snout depressed, sharp, with or without a long filament or spear-like projection that may bear terminal teeth and/or lateral filaments (Castle, 1984). Eye tubular, directed upwards, forwards and outwards. Teeth sharp, the anterior ones generally larger than those behind; one or two prominent teeth on tips of upper and lower jaws. Pigmentation as conspicuous dendritic melanophores on body wall alongside gut; variously on body wall behind anus, midlaterally, on caudal fin, above and below midlateral level, and scattered around brain, along jaws and on rostral filament.

### Type B<sub>10</sub> (Smith and Castle, unpubl.; Fig. 1A-C)

*Diagnosis.* - No rostral filament; lateral pigment present; anus at about midpoint of body; a compact pair of kidneys immediately above gut near anus; pigment patches at regular intervals along gut; two widely spaced melanophores on body wall immediately above anal fin; widely spaced, dendritic melanophores on midlateral myosepta, originating deeply internally and more or less alternating on left and right sides; a few scattered on caudal fin; several minute, deep melanophores below midlateral level in front of caudal; a series along edge of upper jaw. Myomeres 119-134 and maximum observed TL 55 mm ("low count" species) and 136-143 and 68 mm ("high count").

**Identification.** - The "low count" species agrees closely in myomere count with leptocephali of *Dysomma anguillare* Barnard, 1923 from the Atlantic (118-128) as described by Smith (1989). This species occurs widely in the Indo-west Pacific as adults. It agrees with the Atlantic material in overall body form (non-rostrate), but Pacific specimens have well developed lateral pigment, not present in Atlantic *D. anguillare* larvae. The "high count" species is similar in body form and pigmentation but has more myomeres.

**Material studied.** - Type B<sub>10</sub> "low count" (65 specimens): Dana stations 3676 haul II (15 spms), VIII (1); 3678 V (3), VII (5); 3683 VIII (1); 3688 II (1), IV (1); 3689 II (ZMUC P32997, voucher spm), VII (2), XI (1); 3714 II (1); 3715 III (1); 3731 VI (ZMUC P32995, voucher spm), 3731 VI (4); 3733 II (2); 3736 VI (7); 3738 II (1); 3740 II (2); 3745 II (1); 3749 II (1); 3766 XXI (1); 3787 II (3); 3795 II (1), III (1); 3902 II (1); 3903 I (1); Galathea 436 (1); ORSTOM Bora II (1); ORSTOM G1-29-63 (1). Summary: Celebes Sea, Banda Sea, Sulu Sea, Bismark Sea, South China Sea, northeastern and eastern Indian Ocean, western Indian Ocean, southwestern Pacific.

Type B<sub>10</sub> "high count" (56 spms): 3676 II (1); 3683 V (1), VI (1); 3638 VIII (2); 3738 II (2); 3739 II (12), VII (1); 3740 II (15); 3744 II (1); 3745 I (1); 3749 II (2); 3789 II (5), VI (1), VII (2), VIII (3); 3791 II (2); 3795 II (3); ORSTOM Bora III (1). Summary: Sulu Sea, Bismark Sea, northeastern Indian Ocean, southwestern Pacific.

**Reproductive state.** - Twenty of the "low count" species (31.0-68.5 mm total lengths) had ovaries, while 45 (18.0-53.0 mm TL) did not; 14 of the "high count" species (31-65 mm TL) had ovaries, 42 (16.0-70.0 mm TL) did not. The ovaries (Fig. 2A) are tubular structures lying immediately above the gut between myomeres 17-31 to 51-53 ("low count") and between myomeres 22-29 to 57-64 ("high count"), i.e., to just above anus in each case. In smaller (?younger) specimens the front of the ovary lies further back, so presumably development proceeds anteriorly with growth. The two ovaries are separated for most of their length but become appressed to each other near the level of the anus.

In a specimen of 52.0 mm TL ("low count", Fig. 2B-C) there are at least 1,000 ova in each ovary, the largest ovum being about 45  $\mu$ m diameter. A transverse section near the anus shows 4-7 ova across each ovarian section (Fig. 2B-C). The paired kidneys and kidney ducts lie immediately above the ovaries together with what appear to be the smaller, densely packed and staining cells on the dorsomedial aspect of each kidney (Fig. 2D). We suggest that these medial cells constitute haemopoietic tissue. That they might be testicular tissue is a further, though unlikely, possibility.

In the remainder of the 121 specimens ("low" and "high" count species taken together) there is no other evidence of reproductive development. However, about the same number of specimens as that of the females show well developed kidneys and are possibly males. Since the testes develop in association within the tubules of the mesonephric kidney, we might expect there to be some structural distinction in the cells of the kidney but this was not obvious in the sections we made.

#### **Type D<sub>1</sub> (Smith and Castle, unpubl.; Fig. 1D-F)**

**Diagnosis.** - A rostral filament present. Lateral pigment present. Anus at two-thirds of body length; kidney long, ending some distance before anus; pigment patches along whole of gut; no postanal pigment except for a tiny, expanded melanophore close to anus; relatively compact, dendritic melanophores originating deeply and extending onto the midlateral myosepta, a few alternating on left and right sides; about five expanded melanophores on body wall between midlateral line and ventral body wall margin

just before level of anus; two deep melanophores below these; no caudal pigment; three small patches of pigment on rostral filament. Myomeres 127-145; maximum observed size 92.5 mm TL.

*Identification.* - The D<sub>1</sub> type larvae are most closely similar to Type D<sub>4</sub> from Bermuda, with 128-133 myomeres, described as Type VI by Keller (1976), except that the present specimens lack the group of stellate pigment spots above the midlateral level. With the Bermuda material they are provisionally grouped with *Leptocephalus proboscideus* Lea, 1913 with 128-134 myomeres. Smith (1989) suggests that they might either belong to a group of closely related species or to one that is highly variable.

*Material studied.* - Dana st. 3685, haul II (ZMUC P32996, voucher specimen); ORSTOM Bora I, st. 8 (2); Bora III, st. 9 (1); Cyclone II, st. 8 (1); Cyclone III, st. 1 (MNHN 1998-0051, 92.5 mm TL, voucher spm and one other). Summary: Sulu Sea, southwestern Pacific.

*Reproductive state.* - In the large specimen from Cyclone III (92.5 mm TL) that was studied in more detail, the ovaries lie from myomere 24 to 64, i.e., to 16 myomeres before anus, and each contains approximately 4,500 to 6,000 ova.

## DEPTH DISTRIBUTION

Bruun (1937) showed that Atlantic leptocephali of *Synaphobranchus kaupi* Johnson, 1862, and *S. bathybius* Günther, 1877 were most abundant in about 100-275 m. Metamorphosing specimens were very few indeed and these were captured in the greater depths of this range.

The pattern of occurrence in the water column of ilyophine leptocephali in the Indo-west Pacific is similar. The Dana investigations there most often employed a string of simple ring nets of 150-200 cm diameter along the towing wire. These were typically placed at intervals from 50 m to 600 m wire out (m.w.o.), frequently as deep as 1,000 m.w.o. and occasionally much deeper. Surface nets were also employed though no leptocephali were taken in these. Most hauls were made in the period of darkness. Because nets were infrequently deployed at greater actual depths of more than 1,000 m it is not possible to define the deeper limit at which ilyophine larvae might live.

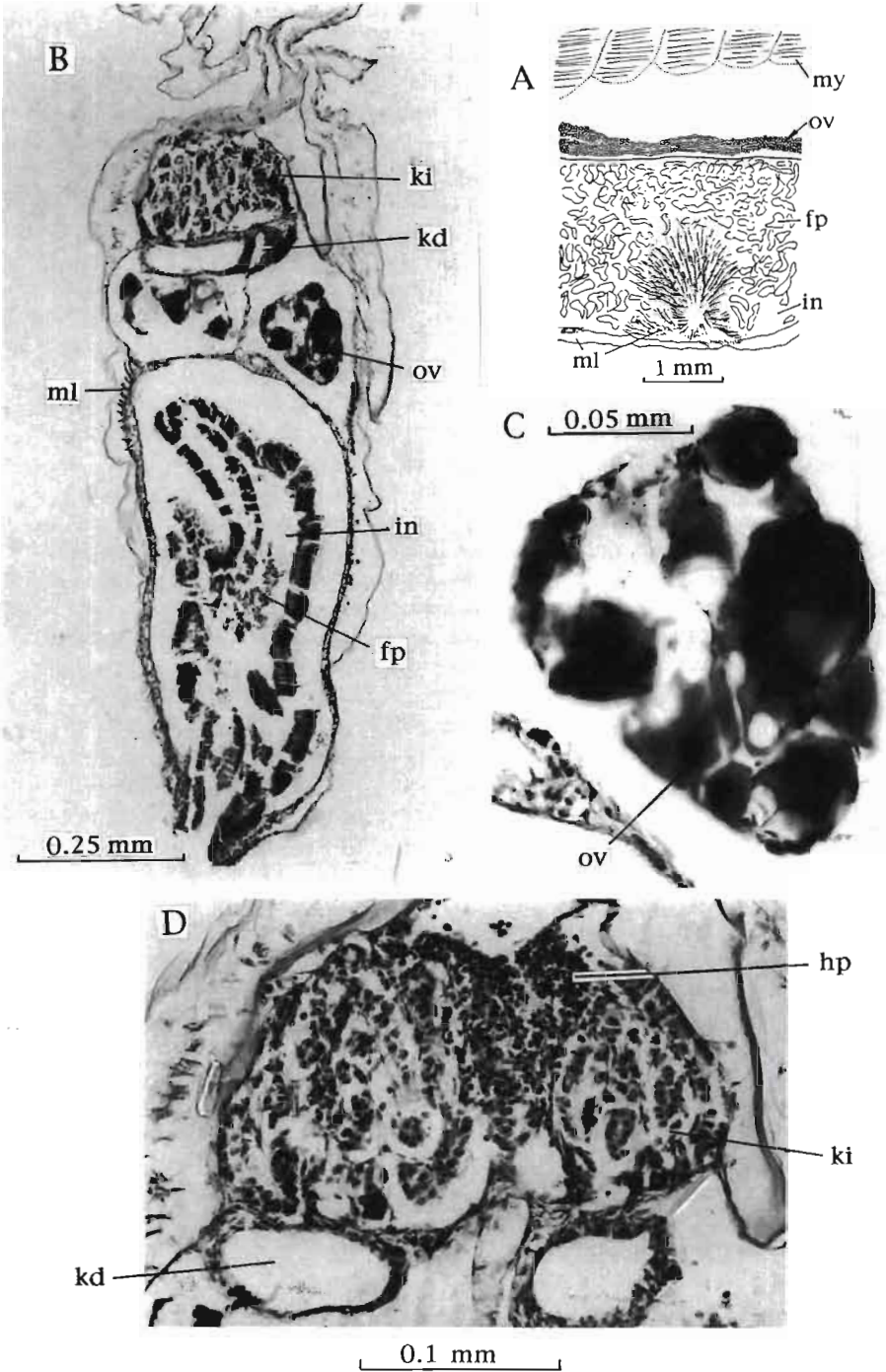
Ilyophine leptocephali first appeared in hauls at 200 m.w.o., though only 21 of the total of 188 larvae were captured in the 628 hauls made between 200 and 500 m.w.o. Most larvae (167) were collected in the 291 hauls with 600 m.w.o. or greater, with the majority (140) actually at 600 m.w.o.

Larvae of several species of *Moringua* (Moringuidae) were also caught in numbers by the Dana throughout the Indo-west Pacific, often at the same station as the ilyophines, though at markedly shallower depths. For example, the 324 moringuid larvae appeared first in hauls at 50 m.w.o., with 321 collected in the 1,016 hauls made between this depth and 500 m.w.o.

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Fig. 2. - **A:** Portion of ventral part of body of Species D<sub>1</sub>, ORSTOM cruise Cyclone III, station I, 95.5 mm TL, MNHN 1998-0051. **B:** Transverse section of ventral third of body of Species B<sub>10</sub>, Dana station 3689, haul II, 52.0 mm TL, ZMUC P32997, voucher specimen. **C:** As above in figure B, ovarian section enlarged. **D:** Kidneys, kidney ducts and medial haematopoietic (?) tissue. Different transverse sections to A-C. fp: food particles; hp: ?haematopoietic tissue; in: intestine; kd: kidney duct; ki: kidney; ml: melanophore; my: myomere; ov: ovary.





## DISCUSSION

The ovigerous Type B<sub>10</sub> leptocephali were principally collected in the central part of the Indo-west Pacific (Celebes, Banda, Sulu and South China Seas - Dana specimens), north of New Caledonia (ORSTOM) and the southwestern and northeastern Indian Ocean (Galathea Expedition and ORSTOM, respectively). The Type D<sub>1</sub> larvae were collected in the Sulu Sea and north of New Caledonia (ORSTOM). Atlantic ilyophine leptocephali, including those reported by Smith (1989), were not available for study.

It is clear from the depth distribution figures presented that ilyophine leptocephali, at least during the hours of darkness, live almost exclusively at about 200 m (600 m.w.o.) while moringuids live nearer the surface at ca. 15-150 m (50-500 m.w.o.). Whether the ilyophines move down further during the day is not known though the presence of the "tubular" eye and in recognition of the depths that they occur in, suggest that they live near the lower limits of where light penetrates.

Blache *et al.* (1970, fig. 14) illustrated a section of a metamorphic specimen of the ilyophine *Nettodarus* sp. from the East Atlantic that clearly showed an ovary with ova. In this specimen the kidney and ovary extended well posterior to the anus. There was, however, no indication in the text or on the figure caption that the authors were aware that the structure was an ovary. Castle (1978) described ovigerous metamorphic leptocephali of the Atlantic nettastomatid *Facciolella oxyrhynchus* (Bellotti, 1883) and the Indo-west Pacific species *F. saurenheloides* (D'Ancona, 1928) and *Facciolella* sp. (South China Sea). Other records of precocious ovarian development, almost all in metamorphic larvae, were also noted then, including that of *Thalassenchelys foliacea* Castle & Raju, 1975 (of unknown family identity) and the saccopharyngiformes *Monognathus taaningi* Bertin, 1938, *Monognathus* sp. and *Cyema atrum* Günther, 1878.

Castle (1978) referred to the remarkable Indo-west Pacific *Schindleria praematura* (Schindleriidae) which also undergoes precocious reproductive development, in that the gonads are developed at 20 mm TL (female) and 15 mm TL (male) while the fish are essentially juveniles rather than larvae. The condition in *Schindleria* is in our view the best described as neotenic, i.e., of adult form in which larval characters have been retained, in the sense of Kollman (1885). In contrast, the ilyophine leptocephali are clearly larvae. The small size of the ova suggests that the specimens are not fully mature, and thus the term paedogenetic, in the sense of von Baer (1866), i.e., larvae in which maturity is reached, though more relevant, cannot strictly be applied. The question of whether pre-metamorphic spawning might take place in these or other eel larvae is an intriguing concept, but is clearly not resolved by our present observations.

The presence of ovaries in the ilyophine leptocephali means that sex has already been established at this early stage though the possibility that the species might be protogynous cannot be discounted. It is therefore likely to be determined genetically rather than environmentally, as has been suggested from time to time for *Anguilla*. Castle and Böhlke (1976) showed that in the Atlantic *Moringua edwardsi* (Jordan & Bollman, 1889) the bimodal distribution of myomere number in leptocephali closely matched the bimodal distribution of vertebral numbers in adult males and females. The two groups of larvae of *M. edwardsi* must therefore have been males and females.

Several novel and significant aspects of the early life history of ilyophine eels have emerged from our study, though it remains to be seen how general these might apply to ilyophines as a whole.

From the capture and larval body size information it is quite clear that ilyophines must spend their early lives, i.e., from at least as small as 16 mm to full larval growth of about 65 mm, some distance from the surface. Most of the 200 study specimens were captured at about 200 m in the hours of darkness, but because the Dana nets were seldom deployed during daylight it is not possible to say whether the leptocephali then move deeper, or more unlikely, approach the surface. The possession of the "tubular" eye lends support to the conclusion that they indeed live at some depth. This is the first time that it has been possible to pinpoint the depth preference of leptocephali, including also those of the near-surface *Moringua*. The almost total lack of metamorphic larvae further suggests that ilyophine leptocephali move to greater depths for this stage to take place and development of the ova to be completed. Presumably then, deeper dwelling, maturing adults must move upwards to spawn in shallower depths where food of suitable type and abundance is available to the larvae.

The presence of ova in what are undoubted larvae as small as 32 mm is an extraordinary observation and to our knowledge is unprecedented in fishes. However, that it is not restricted to ilyophines amongst the eels is shown by the record of it in the nettastomatid *Facciolella* sp. (Castle, 1978). Even more remarkably, developing ovaries are known also to occur in leptocephali as small as 20 mm TL, as recently discovered in a specimen of the eel family Chlopsidae presently being studied by the senior author as this paper went to press.

**Acknowledgements.** - We thank A. Hoverd and T. Andrews, Victoria University of Wellington, for preparing the histological sections and assistance with photography, respectively, and J. Dawson for the French abstract translation. The senior author also thanks the Internal Research Committee and the Leave Committee, VUW for providing funds for research assistance and travel.

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## SWIMMING PERFORMANCE OF LATE PELAGIC LARVAE OF CORAL-REEF FISHES: *IN SITU* AND LABORATORY-BASED MEASUREMENTS

by

Jeffrey M. LEIS (1) & Ilona C. STOBUTZKI (2)

**ABSTRACT.** - Two complementary methods provide insights into swimming performance of coral-reef fish larvae of 15 families. Laboratory swimming chamber measurements and *in situ* observation of larvae by divers supply data on swimming distance (or, duration) and speed, respectively. These late larvae are strong, effective swimmers, capable of speeds higher than ambient currents and capable of swimming non-stop for tens of km over tens of hours. We found a strong positive correlation between speed and distance swum both among species and among families. However, the relationship was not significant within either the Chaetodontidae (6 spp.) or the Pomacentridae (8 spp.) alone, probably due to the low number of species for which we had data on both variables and the narrow range of speeds and distances measured within each family. Not all species are amenable to measurement by both methods, and the methods differ in advantages and disadvantages as well as in the data they provide. The exceptional swimming performance we document requires reassessment of views on dispersal and retention of reef-fish larvae.

**RÉSUMÉ.** - Performances de nage des larves pélagiques âgées de poissons récifaux: mesures *in situ* et en laboratoire.

Deux méthodes complémentaires ont apporté des données sur les performances de nage des larves de poissons de récif appartenant à 15 familles. Les mesures en laboratoire et les observations *in situ* des larves par des plongeurs ont permis de recueillir des données sur la distance (ou la durée) et la vitesse de nage. Les larves pélagiques âgées sont robustes, bonnes nageuses, capables de nager à des vitesses supérieures à celles des courants ambiants, sur des distances de plusieurs dizaines de km ou pendant plusieurs heures sans s'arrêter. Nous avons trouvé une forte corrélation positive entre la vitesse et la distance parcourue entre espèces et entre familles, sauf pour les Chaetodontidae (6 espèces) et les Pomacentridae (8 espèces), probablement en raison, d'une part, du nombre réduit d'espèces pour lesquelles les données étaient disponibles et, d'autre part, des gammes étroites des vitesses et des distances mesurées pour chaque famille. Toutes les espèces ne peuvent être soumises à ces deux types de mesure qui diffèrent par leurs avantages et leurs inconvénients, et par les données qu'elles fournissent. Les exceptionnelles performances de nage observées nous obligent à réviser nos concepts sur la dispersion et la sédentarité des larves des poissons de récif.

**Key-words.** - Coral-reef fishes, Larvae, Swimming distance, Swimming duration, Swimming speed, Dispersal, Retention.

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Bony fishes that live on coral reefs have a bipartite life history, as do most marine animals (Leis, 1991). The adults live on the reef and are relatively sedentary, but when they reproduce, their propagules must fend for themselves in pelagic environments ranging from atoll lagoons to the open ocean. During the pelagic phase, larvae may travel far from their natal reef, but at the end of this phase, they must find a coral reef upon which to settle and complete their life cycle. When they first enter the pelagic environment, these reef-fish propagules are small (1-2 mm), poorly developed and weakly swimming (Leis, 1991), but by the time they return to the reef 10 to 100 days later, they are small, well-developed fishes (normally 1-3 cm SL)<sup>(3)</sup>, and their fins are formed, or nearly formed, and functional (Leis and Rennis, 1983). Thus, at this point, if swimming performance is correlated with morphological development, they are at least potentially capable of effective swimming (by effective swimming, we mean swimming at speeds at least as great as those of the ambient currents). Knowledge of the amount of control over position and trajectory achieved by these small settlement-stage fishes is critical for understanding how they find the reef, and how much settlement choice they have. In other words, are reefs encountered by these larvae passively, and primarily by chance, or are they found by effective swimming, and in a directed way? Of course, they might also be found by chance by undirected effective swimming.

Until recently, most reef-fish ecologists and fishery biologists accepted perspectives developed for larvae of invertebrates and considered that pelagic larval stages were not effective swimmers (e.g., Williams *et al.*, 1984; Roberts, 1997), and that reefs were found primarily by chance. This view seemed to be supported by the swimming performance of temperate fish larvae (Blaxter, 1986; Miller *et al.*, 1988; Brietberg *et al.*, 1995). However, larval fishes and their capabilities are not necessarily the same as those of larval invertebrates (Leis, 1991; Boehlert, 1996; Cowen and Sponaugle, 1997), nor, it turns out, are temperate fish larvae necessarily the same as reef-fish larvae even when comparisons are not confounded by large taxonomic differences (Leis and Carson-Ewart, 1997). This recognition combined with a number of anomalies in the distribution of reef-fish larvae led to conclusions that behaviour of larvae was an important determinant of distributions and trajectories during the pelagic period (Leis, 1991; Boehlert *et al.*, 1994; Cowen and Castro, 1994). However, direct evidence was lacking and there was little more than conjecture about what kind of behaviour was involved. Recent work on the swimming capabilities of reef-fish larvae near to settlement has led to a reassessment (Stobutzki and Bellwood, 1994, 1997; Leis *et al.*, 1996; Leis and Carson-Ewart, 1997). It is now clear that many reef-fish larvae near the end of the pelagic stage are not only capable of effective swimming, but are also capable of impressively long swimming bouts and of covering considerable distances.

Insight into swimming capabilities has been arrived at by two different, but complementary, methodologies (Stobutzki and Bellwood, 1994, 1997; Leis *et al.*, 1996; Leis and Carson-Ewart, 1997). Our purpose here is to review and compare these new data, detail the complementarity of our respective approaches and place all of this in perspective.

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(3) We seek to avoid arguments over terminology: depending on the species and the system of terminology adopted, reef fishes near the end of the pelagic phase may be considered larvae, juveniles or either (Leis and Trnski, 1989; Leis, 1991). The important point for our purpose here is that they are still pelagic, and we refer to them all as larvae for convenience.

## METHODS

We used late-stage (= settlement-stage) larvae captured in the field, primarily with light traps (Stobutzki and Bellwood, 1997), although some larvae were captured with crest nets. Only brief descriptions of the methodologies are given here: refer to the cited papers for details. The data on swimming performance considered here are from our papers cited below, and Stobutzki (1998).

Stobutzki's approach has been laboratory-based, and utilised a multi-channel swimming chamber or flume (Stobutzki and Bellwood, 1994, 1997). For measurement of relative swimming abilities (Stobutzki and Bellwood, 1994), flow rate in the chamber was increased stepwise to determine the greatest swimming speed that the fish could maintain for five minutes (i.e., critical swimming speed of Beamish, 1978). For measurements of sustained swimming abilities (Stobutzki and Bellwood, 1997), the speed in the chamber was fixed at the average current speed in the area (13.5 cm/s), and the fish allowed to swim until exhaustion. This enables the swimming duration and equivalent distance travelled to be determined. Frequently, fish swam for several days, and because they were not fed, this provides conservative estimates of swimming performance.

Leis' approach has been field-based, and involved pairs of divers releasing larvae individually in the ocean and following them with a modified plankton-net flowmeter and stopwatch, measuring distance swum and time elapsed *in situ* (Leis *et al.*, 1996; Leis and Carson-Ewart, 1997). Normally, this was done for 10 min, a length of time that balances logistic requirements with a reasonable distance travelled for calculation of speed.

## RESULTS AND DISCUSSION

### Taxa

Light traps are selective (Doherty, 1987; Choat *et al.*, 1993), so the majority of our data are from pomacentrids, lethinids, apogonids and chaetodontids. Crest and channel nets provide a wider variety of species with which to work (Leis, unpubl. data), but these nets require particular reef morphology and tide regimes which are not available everywhere. Also, turbulent conditions in the net may damage or fatigue larvae. Further, such nets must be used in close proximity to the reef (often over it), raising questions about the source of the catch (e.g., are the fishes truly pelagic?). Reared larvae could be used by either laboratory or field approach. Whereas this offers several advantages, it may present difficulties due to confounding factors that may influence swimming performance such as diet and light regimes. It also remains to be shown that reared and wild larvae have equivalent swimming performance (Taylor and McPhail, 1985; Duthie, 1987; Ennis, 1995).

Beyond the selectivity of the capture method, not all species are suitable for use with both approaches. There are some morphological reasons for this, but most are behavioural. For example, serranids (e.g., *Plectropomus*, *Diploprion*) could not be induced to swim in the chamber. In contrast, these serranids are suitable for *in situ* measurements. Several taxa, particularly *Pomacentrus* spp. ordinarily will not swim when released *in situ* in open water, but drift to the soft bottom below, or are attracted to the divers. However, these pomacentrids do swim well in the chamber. Some holocentrid, blennioid and acanthurid species, among others, seem to regard the divers as potential settlement sites when released, and will attempt to find a hiding place, even if it is amongst the divers' gear.

Holocentrids and acanthurids swam successfully in the chamber, however. Finally, a few species, notably tetraodontids, will interrupt short bouts of swimming with periods of hovering and facing the divers. Some of these species will swim well in the chamber. Fortunately, most taxa are suitable for at least one approach.

### **Different types of information from each approach**

Although our two approaches to investigating swimming performance of the late larvae of coral-reef fishes are in many ways complementary, it is important to emphasise the different information each provides. The *in situ* approach provides information on the speed at which the larvae choose to swim in the field over short periods (typically, 10 min). This is not the maximum speed of which the larvae are capable, either in a burst or sustained sense. The speeds achieved by larvae when pursued either by predators or by divers attempting to obtain voucher specimens are much higher, as are maximum speeds (i.e., the critical speed) obtained with the swimming chamber. In contrast, the laboratory swimming chamber approach gives critical swimming speed (i.e., the maximum speed the larvae can maintain for five minutes), or duration of swimming (i.e., the time over which a fish can maintain a fixed speed and the distance swum during that time).

The results are complementary in several senses. The speeds at which the majority of taxa choose to swim *in situ* are in excess of those used in the swimming chamber, and this indicates that swimming duration estimates are realistic, or perhaps, conservative. Conversely, the fact that these taxa can swim at  $> 10$  cm/s for several days in the chamber, indicates that the 10-min long *in situ* measurements are indeed sustained speeds, not burst speeds.

*In situ* swimming speeds of 43 of the 50 species (15 families) measured by Leis and Carson-Ewart (1997) were greater than the average current speed in the study area, and, overall, averaged 20.6 cm/s. Therefore, the majority of these species were effective swimmers.

Mean swimming distance of the 51 species (9 families) measured by Stobutzki and Bellwood (1997) in the laboratory swimming chamber at the average ambient current speed was 40.7 km, and family means ranged from 3.6 to 94.4 km. This is equivalent to an overall mean duration of 83.7 h, and family means of 7.4 to 194 h. The Great Barrier Reef is only about 50 km wide where most of our work was done, so it is obvious that late-stage larvae have the potential to exercise considerable control over their settlement site.

The greatest advantages of the *in situ* approach are that data are derived under pelagic field conditions, and the larvae themselves select the swimming speed. However, this can only be done during the day, and "blue water" diving presents many challenges. It is also labour-intensive, limited to observations of one larva at a time, and provides swimming data over relatively limited periods. The presence of the divers may introduce unknown biases, even when the larvae appear to be behaving naturally. In the pelagic environment, a reference point is frequently absent. In spite of this, for each species, standard errors of *in situ* mean swimming speeds are relatively narrow (Leis and Carson-Ewart, 1997), so speeds do seem to be regulated. This raises the question of how this is achieved: perhaps the lateral-line system is involved.

In contrast, the laboratory swimming chamber can be used both day and night and several individuals can be tested at one time: in fact, once set up, it is nearly automated. It can measure swimming performance over much longer time periods than can the *in situ* approach, and will allow determination of the distances fish can travel at a range of



speeds. As with all laboratory-based systems, there are possible, unknown biases, and the decision on test speeds to be used is not always clear-cut (in work to date, the average ambient current speed has been used), but conditions are much easier to control. In a swimming chamber, in contrast to pelagic habitat, the fish always have at least a visual reference point, and can assess their swimming performance accordingly. The swimming chamber is useful for obtaining rapid, comparative measures to determine the effect on swimming of factors such as temperature or diet, or even for comparisons between captive reared and wild individuals.

### Direct comparisons between the two approaches

Comparison of swimming performance measured by our two methods reveals that *in situ* swimming speed and laboratory-determined swimming distance (or duration) are strongly correlated. At a family level, we can compare eight families for which we both have information, and there is a strong, positive correlation ( $r = 0.87$ ,  $p = 0.005$ : see Fig. 1 for regression details) between mean speed and mean distance. If the comparison is at a level below family (4 taxa at generic level and 16 taxa at specific level), the correlation is lower, although "more significant" due to higher "n" ( $r = 0.77$ ,  $p < 0.001$ : Fig. 2), and the regression line explains less of the variation. However, much the same relationship exists at both species and family level, because slopes and intercepts are similar. In contrast, within each of the two best-represented families (Pomacentridae and Chaetodontidae), the relationship is positive, but it is significant for neither family. This is probably due to the small number of species for which we had data on both speed and distance (8 and 6 spp., respectively) and the relatively narrow range of values within each family. Within-family variations in both speed and duration are in some cases relatively large, reflecting both real differences among species and variation among individuals of a species. The fact that the relationship between speed and distance explained less of the variation at the species level than at the family level is a result of such variations, particularly the former. Also, the family mean values for the two methods were based on different mixes of species. Thus, some caution is required in any application of the relationships we describe here.

What does a strong positive relationship between these two independently-derived measures of swimming performance mean? At the most basic level, it indicates that strongly swimming taxa can be characterised as such no matter which measure of swimming performance is used. It might also be possible to predict one measure if the other is known: for example in a species that is not suitable for one of the methodologies. The swimming chamber data were all gathered at the same speed, so we do not know if swimming duration (i.e., time) and distance swum are independent. For example, if species with *in situ* speeds  $> 13.5$  cm/s, were tested in the swimming chamber at their *in situ* speed rather than the standard 13.5 cm/s (see Methods) they might either swim the same distance in a shorter time, swim a greater distance in the same time, or perform in an intermediate way. This highlights the need in future research for swimming-chamber-based measurements at different speeds to determine how distance swum is affected.

The laboratory swimming chamber results show that late-stage larvae of at least some taxa (e.g., apogonids, nemipterids) can swim for hours at a time at speeds higher than those they choose *in situ* over short periods. This is a further indication of the conservative nature of the *in situ* swimming speed estimates.

Direct comparisons of critical swimming speeds with *in situ* swimming speeds are not particularly meaningful. The former measure how fast fish can swim in a particular

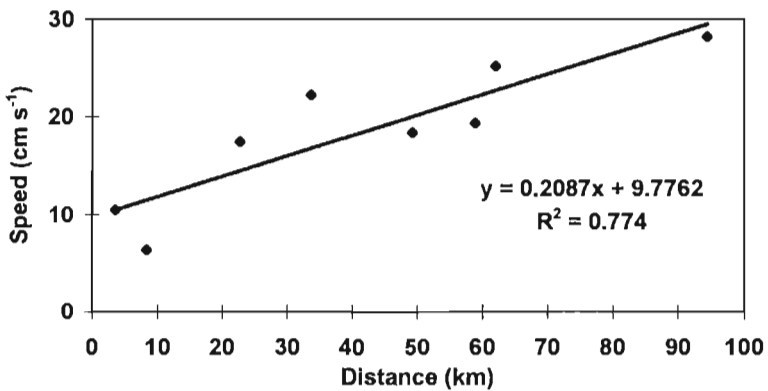


Fig. 1. - Relationship between *in situ* swimming speed and distance swum in the laboratory swimming chamber based on means for the eight families for which both measures were available. The regression is significant at  $P = 0.005$ . Families included are (from low to high distance swum): Nemipteridae, Apogonidae, Pomacanthidae, Pomacentridae, Chaetodontidae, Lethrinidae, Lutjanidae and Acanthuridae. Swimming distance of 100 km is equivalent to swimming duration of 206 h.

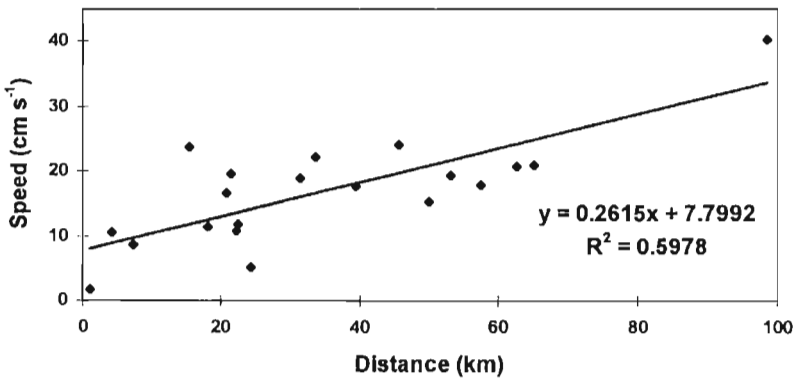


Fig. 2. - Relationship between *in situ* swimming speed and distance swum in the laboratory swimming chamber based on means for the 20 individual taxa for which both measures were available. The regression is significant at  $P < 0.001$ . Taxa included are (from low to high distance swum): *Apogon taeniophorus*, *Scolopsis bilineatus*, *Dischistodus* sp., *Chromis atripectoralis/viridis*, *Chrysiptera rollandi*, *Neopomacentrus cyanomos* (?), *Dascyllus aruanus*, *Pomacentrus moluccensis*, *P. amboinensis*, *Apogon trimaculatus*, *Neopomacentrus azysron*, *Pomacanthus sexstriatus*, *Chelmon rostratus*, *Chaetodon plebeius*, *C. trifascialis*, *Lethrinus* sp., *Chaetodon aureofasciatus*, *C. rainfordi*, *C. trifasciatus* and *Acanthurus* sp. Swimming distance of 100 km is equivalent to swimming duration of 206 h.

laboratory situation, the latter measure how fast fish choose to swim in the field. By design, critical swimming speeds will always be greater than *in situ* swimming speeds. However, comparisons among critical swimming speeds can provide a useful index of relative performance.

## CONCLUSIONS

The *in situ* and laboratory swimming chamber approaches to investigating swimming performance of late larvae of coral-reef fishes each provide different types of data: swimming speed and distance (or duration) swum, respectively. Because of their differences, they are in many ways complementary and provide valuable perspective to each other. The swimming chamber approach is best suited for measurements over long (hours to days) periods, for comparisons of stamina at a range of speeds, or for rapid measures of relative abilities. The *in situ* approach is best suited for measurements over short periods (minutes), but allows simultaneous observations of little-known aspects of larval fish behaviour in the field including orientation, depth selection, feeding, predation, and interaction with predators and other pelagic animals (e.g., see Leis *et al.*, 1996; Leis and Carson-Ewart, 1998).

Both approaches have shown that the late larvae of many species of coral-reef fishes are remarkably strong swimmers, in terms of both speed and distance swum. Although species that have larger larvae swim faster and further (Stobutzki and Bellwood, 1997; Leis and Carson-Ewart, 1997), late larvae are strong swimmers regardless of size. Swimming performance of coral-reef fish larvae is markedly superior to that of temperate fish larvae of similar size, not only overall, but also when comparisons are confined to within the same Order (Leis and Carson-Ewart, 1997). Even in isolation, these results show that late-stage larvae of coral-reef fishes can cover large distances (tens of km) in their search for suitable settlement habitat, and that they can do so quickly and at speeds that can counteract ambient currents. This alone increases their chances of encountering suitable habitat, as compared to passively drifting larvae, even if no assumption is made about orientation and swimming direction. It is now clear that late-stage larvae of many species of coral-reef fishes are anything but passive (contra Roberts, 1997), but we need to obtain data on other, as yet untested, taxa (e.g., Blenniidae, Eels, Gobiidae, Labridae, Scaridae) to determine how wide-spread strong swimming performance is. We next need to ask at what state of development do pelagic larvae cease being passive plankton and start being effectively swimming nekton. The answer is likely to differ among species.

Our studies on orientation seem to indicate that late-stage larvae can detect reefs at a distance (Leis *et al.*, 1996; Stobutzki and Bellwood, 1998). If this is verified, the combination of swimming and orientation abilities possessed by many late larvae of coral-reef fishes would mean they have the potential to control position and trajectory in the pelagic environment and to select settlement reefs to a much greater degree than previously thought realistic. This will require a major re-assessment of our concepts of dispersal and retention during the pelagic stage of reef fishes.

**Acknowledgements.** - We thank staff at the Lizard Island Research Station and EVAAM Rangiroa for making our research programs possible and our stays enjoyable. Our thanks, also, to all the people who assisted us in the field and laboratory. This work was supported by ARC Grant A19530997 and DIST grant 94/2743 to JML, funding supplied by EPHE during JML's tenure as visiting Professor at EPHE, Université de Perpignan, and by MRG and MRA from James Cook University, a GBRMPA Augmentative Grant, a Lizard Island Research Station Doctoral Fellowship and an AFUW Bursary to ICS. We thank S. Bullock who assisted editorially, and L. Fuiman who constructively criticised the manuscript and suggested the phrase "effective swimming". This is a contribution from the Lizard Island Research Station and EPHE-CRIOBE.

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## **A METHOD TO TAKE PHOTOGRAPHS OF LIVE LATE LARVAL AND JUVENILE REEF FISHES**

by

Akinobu NAKAZONO (1)

**ABSTRACT.** - A new method to photograph late larvae or juveniles of reef fishes in the field immediately after collection by hand nets was developed. In this method, an extension tube was mounted on a Nikonos V underwater camera and a small glass case was also mounted at the focus distance of the lens. Collected juveniles were placed in the case and a strobe with TTL (Through-The-Lens) exposure metering provided illumination. Photographs taken with this method provided satisfactory detail of live juvenile color patterns.

**RÉSUMÉ.** - Une méthode pour photographier les stades larvaires avancés et les juvéniles de poissons récifaux vivants.

Une nouvelle méthode pour photographier les stades larvaires avancés et les jeunes poissons récifaux, immédiatement après leur capture à l'épuisette, est décrite. Le matériel utilisé pour cette méthode photographique comprend un tube-allonge monté sur un appareil étanche Nikonos V et une petite chambre de verre montée à l'extrémité du tube à la distance focale de l'objectif. Les jeunes poissons récoltés ont été placés dans la chambre de verre et un flash en mode d'exposition TTL (mesure à travers l'objectif) a été utilisé pour fournir la lumière nécessaire à la prise de vue. Les photographies prises avec cette méthode présentent un bon rendu des détails et des couleurs des jeunes poissons.

**Key-words.** - Reef fishes, Larvae, Juveniles, Photography.

In studies of larval and juvenile fish morphology, dead specimens collected by nets are usually examined. Specimens are fixed with formalin or ethanol after collection. Because of this, skin pigmentation, especially blue, yellow and red pigments, disappear. Observation of live specimens immediately after collection is often attempted but is frequently unsuccessful because the small delicate fishes are damaged during net towing, usually over a long period of time, or during the course of transfer from the net to a bucket or a bottle. Many juveniles with intriguing coloration are observed while diving but descriptions of their body color in life are relatively rare. The usual method for taking photographs of juveniles of reef fishes is to use a camera mounted with a macro-lens in an underwater housing. The lens usually has a long focal length of 100-200 mm. This method, however, requires considerable patience while the observer waits for juvenile fishes to come into focusing range. Also, it is not easy to approach juveniles until they came into the focus range of a big underwater camera or housing. Furthermore, the juveniles will sometimes retreat deep into a hole in the reef and will not appear for a long period of time if an observer with sound-producing scuba equipment is nearby. To over-

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come these problems, a new method to photograph live larvae or juveniles underwater immediately after collection by hand nets was developed and the method was tested on several juvenile reef fishes.

## MATERIAL AND METHODS

The idea of this method comes from a simple modification of a technique by Randall (1961). A small transparent glass case was attached to the front of a close-up extension tube (No. 35-M1, Sea & Sea Co. Ltd.) mounted on a Nikonos V underwater camera equipped with a 35 mm Nikonos lens. This glass case measured 50 x 40 mm and was slightly larger than the visual field at the focal range (35 x 24 mm). The depth of the case was 5 mm. The case was attached with adhesive to the focusing frame showing the range of the field. The top of the case had an opening that could be closed with a lid made of transparent plastic (Fig. 1A). Juvenile fishes could be inserted into the case through this opening and the top closed to prevent their escape. Reef fish juveniles, found while diving, were collected with one or two hand nets and placed in the glass case immediately. A Nikon SB105 strobe with automatic TTL (Through-The-Lens) exposure control provided illumination.

## RESULTS AND DISCUSSION

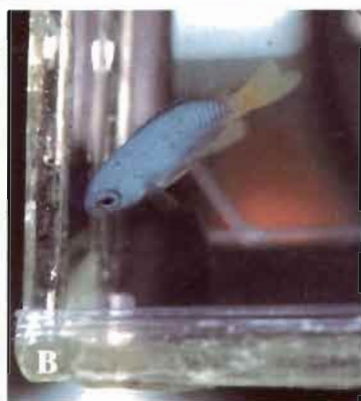
At Makurazaki, Kagoshima Prefecture, Japan, a 14 mm TL juvenile of neon damsel, *Pomacentrus coelestis* Jordan & Starks, 1901 (Pomacentridae) was collected with a hand net on September 20, 1997. The fish was placed carefully in the glass case underwater and the top lid was closed. For a comparison between the new method and one used previously, the fish was photographed from the opposite side of the glass case with another camera mounted in an underwater housing with 90 mm autofocus lens (Fig. 1B). Then, the same fish was photographed with the new method as the fish swam nearly in the center of the case. Exposures were made at f11-16 on ASA 100 color reversal film (Fig. 1C). After several exposures were made, the juvenile was released where it was collected.

In another test, juveniles of spotty-tail, *Acanthochromis polyacanthus* (Bleeker, 1855) (Pomacentridae), of about 23 mm TL, were collected on October 13, 1997 at Bunaken National Park, Manado, North Sulawesi, Indonesia, with hand nets. An example of photograph taken of this species is given in figure 1D. The figure clearly indicates the success of this new method. Delicate color patterns, which are difficult to see in fixed specimens, are well recorded. Photographs taken from a right angle provide fin ray or scale numbers that are countable. The 35 mm lens and extension tube arrangement pro-

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Fig. 1. - A: Photograph of Nikonos underwater camera with the extension tube (ET) and a glass case (GC). B: Photograph of a juvenile *Pomacentrus coelestis* at Makurazaki, Kagoshima Prefecture, Japan, on September 20, 1997. The juvenile was placed in the glass case and the photograph was taken from the opposite side with another camera mounted in a standard underwater housing. C: The same fish as in B, photographed with the new method. The size of the fish is 14 mm TL. D: *Acanthochromis polyacanthus* juvenile photographed with the new method at Bunaken National Park, Manado, North Sulawesi, Indonesia, on October 13, 1997. The size of the fish is 23 mm TL.





vided on film a fish image of nearly the same size as the specimen. Thus, total length of a juvenile could be estimated reliably by measuring the size of the fish on the photograph.

This method is useful for taking photographs of live larvae and juveniles immediately after collection and for recording coloration patterns of juveniles as they are found in their habitat. This method will have utility in both taxonomic studies of juvenile reef fishes and in understanding the ecological significance of juvenile color patterns.

**Acknowledgements.** - The author is greatly indebted to Dr. Janny D. Kusen and his students for arranging diving at Bunaken and for help in collecting juvenile fish. I am grateful to Dr. Terry J. Donaldson and anonymous reviewers for critically reviewing the manuscript.

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## MINIREVIEW. THE MICROPYLE OF TELEOST FISH EGGS: MORPHOLOGICAL AND FUNCTIONAL ASPECTS

by

Rüdiger RIEHL (1)

**ABSTRACT.** - All eggs of teleost fishes are all surrounded by an acellular envelope called the zona radiata that presents an impenetrable barrier for sperm cells. In addition, apart from two known exceptions in *Lepidogalaxias salamandroides* (Lepidogalaxiidae) and *Gambusia affinis* (Poeciliidae), none of the teleost spermatozoa have acrosomes. Acrosomes contain substances which lyse the various membranous egg envelopes, and they aid penetration of the genetic material. Because of the absence of an acrosome, another mechanism for the entry of the sperm into the egg evolved to ensure fertilization. Entry is achieved by a preformed perforation in the envelope, the micropyle. All teleost fishes investigated here to fore, have only one micropyle. In sturgeons, for example, there may be as many as 52 of these pores. To date four different types of micropyles have been described. The micropyle has different functions, namely: 1) penetration of the spermatozoa; 2) prevention of polyspermy, because polyspermy leads to abnormal development in teleosts; 3) attraction of spermatozoa by pheromones; and 4) influx of water during the formation of the perivitelline space. It has been shown recently in the medaka (*Oryzias latipes*, Oryziidae) and in the Schneider (*Alburnoides bipunctatus*, Cyprinidae) that the micropyle is closed by a plug of cytoplasm immediately after the first spermatozoon has passed through it. Spermatozoa are able to find the micropyle in four different ways: 1) by accident, this is the simplest way, because there are immense quantities of spermatozoa; 2) by attraction of pheromones; 3) by producing sperm trails as was found recently in Gobiidae and Blenniidae; and 4) by sperm guiding systems as recently described in Cyprinidae, Loricariidae, and Anabantoidei.

**RÉSUMÉ.** - Brève revue. Le micropyle des oeufs des poissons téléostéens: aspects morphologiques et fonctionnels.

Tous les oeufs de téléostéens sont entourés d'une enveloppe non cellulaire appelée la *zona radiata* qui constitue une barrière impénétrable pour les spermatozoïdes. De plus, les spermatozoïdes des téléostéens ne possèdent pas d'acrosomes à l'exception de deux cas bien connus: *Lepidogalaxias salamandroides* (Lepidogalaxiidae) et *Gambusia affinis* (Poeciliidae). Les acrosomes contiennent des substances qui lysent les différentes membranes enveloppant l'oeuf, et ils favorisent la pénétration du matériel génétique. L'absence d'acrosome a favorisé l'évolution d'un autre mécanisme assurant la fertilisation de l'oeuf. Le spermatozoïde entre par une perforation préexistante sur l'enveloppe de l'oeuf, le micropyle. Tous les oeufs de téléostéens examinés jusqu'à présent ont un seul micropyle, mais chez les oeufs d'esturgeons au contraire, on peut trouver jusqu'à 52 pores. Quatre types de micropyles ont été décrits jusqu'à présent. Les fonctions du micropyle sont: 1) permettre la pénétration du spermatozoïde, 2) éviter la polyspermie qui, chez les téléostéens, aboutit à un développement anormal, 3) attirer les spermatozoïdes par des phéromones, et 4) permettre l'entrée d'eau pendant la formation de l'espace périvitellin. Des résultats récents ont montré que, chez le médaka *Oryzias latipes* (Oryziidae) et *Alburnoides bipunctatus* (Cyprinidae), le micropyle est obturé par un bouchon cytoplasmique dès que le premier spermatozoïde est passé. Les spermatozoïdes trouvent le micropyle par quatre moyens: 1) par hasard, la voie la plus simple puisqu'il y a beaucoup de spermatozoïdes, 2) par l'attraction des phéromones, 3) par la formation de traînées de sperme, comme cela a été récemment

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montré chez les Gobiidae et les Blenniidae, et 4) par des systèmes de guidage du sperme comme cela a été récemment décrit chez les Cyprinidae, les Loricariidae et les Anabantoidei.

**Key-words.** - Teleostei, Egg, Oocyte, Micropyle, Fertilization, Sperm guiding system, Zona radiata.

The micropyle of teleosts was first described by von Baer (1835). It was reinvestigated by Doyère in 1849 (cited in Ransom, 1867), who recognized its probable role in fertilization but did not have any experimental evidence for its role. Ransom (1867) experimentally demonstrated the function of the micropyle (compare Wourms, 1997). Since its discovery the micropyle has been found in more than 200 teleost species up to date (Riehl, review in preparation). Many of these papers were published between 1855 and 1920 (e.g., Bruch, 1856; Buchholz, 1863; Owsjannikow, 1885; Eigenmann, 1890 with important studies; for first reviews see Korschelt and Heider, 1902; Hertwig, 1906). In the late 1940s Japanese scientists began to work again on the fish micropyle (Kano, 1949; K. Yamamoto, 1951, 1952; T.S. Yamamoto, 1955). Riehl and Götting (1974) gave the first enumeration of the widespread literature on micropyles. In the last 25 years a couple of papers were published on different aspects of the micropyle in terms of morphology, physiology, function, etc. (for review see Laale, 1980; Guaraya, 1986; Hart, 1990; Riehl, 1991). The aim of this paper is to give a short review of morphological and functional data on the teleost micropyle worked out in these last 25 years.

## EGG ENVELOPE

Fish eggs normally are spherical. An enlarged nucleus with varying numbers of nucleoli and chromosomes is a prominent feature of the central cytoplasm. The latter contains a great deal of yolk. The peripheral regions show vacuoles that are largely responsible for the formation of the perivitelline space, a space that separates the yolk mass from the envelope, after the egg has been fertilized. Externally, the egg is limited by a thin membrane, the oolemma. An envelope of variable dimensions, the zona radiata, covers the oolemma. Normally the zona radiata consists of two layers, an inner zona radiata interna and an outer zona radiata externa. The chemical composition of the interna is known to be largely proteinaceous. Neutral and acid mucopolysaccharides are the main components of the externa (Riehl, 1977a; Patzner and Glechner, 1996).

The envelope serves multiple functions in many but not all species. The main function is the protection of the developing embryo. The zona radiata interna is largely responsible for this protective function. This layer hardens after the eggs have been laid and it is known to increase its tensile strength up to a thousandfold (Lönning *et al.*, 1984; Groot and Alderdice, 1985; Hagenmaier, 1995).

The thickness of the zona radiata varies with ecological challenges and its morphology is indicative of the ecological conditions during spawning and egg development. Normally, the zona radiata has a thickness of 5 to 15  $\mu\text{m}$  but it may be as thick as 60  $\mu\text{m}$  in species that deposit their eggs in pebble beds (e.g., salmonids; Groot and Alderdice, 1985). Eggs that are deposited in the intertidal wave zones may attain a thickness of as much as 100  $\mu\text{m}$  (e.g., hooknose *Agonus cataphractus*; Götting, 1964; verified by the author). Eggs of teleosts that are protected from mechanical forces are normally much thinner. Species that are mouthbreeder or have living offspring have a thin

zona radiata. In the viviparous goodeids and poeciliids the zona is only about 0.5 to 2  $\mu\text{m}$  thick (Riehl and Greven, 1993).

Another important function of the egg envelope is the fixation of the spawned eggs to a substrate, a process that is largely mediated by the zona radiata externa (compare Riehl, 1996; Riehl and Patzner, 1998). There are three main reasons for this action, namely: 1) to attach the eggs in locations that have the optimal environmental conditions for the particular species and also prevent the removal of the eggs by currents or wave actions (Götting, 1964); 2) the deposition in nest-like egg colonies. By placing the eggs in a rather limited space, they can be protected by guarding parents and stand a much better chance of survival (e.g., in cichlids) (Fryer and Iles, 1972); and 3) an improved supply of oxygen. Fish species that inhabit bodies of water with low oxygen supplies may attach their eggs to plant parts immediately below the surface and achieve a more efficient exposure of the eggs by placing them close to the better aerated surface layers. The eggs of the pikes and many cyprinids are deposited in this way (Hegemann, 1964; Steffens, 1980; Riehl and Patzner, 1992).

In some fish species, e.g., in the cyprinids *Tribolodon hakonensis* and *Cyprinus carpio* and in the plecoglossid *Plecoglossus altivelis*, an additional function of the envelope was recently discovered by Japanese workers (Kudo and Inoue, 1986, 1989; Kudo *et al.*, 1988). They found that the mechanically protecting envelope is surrounded by a delicate layer that provides a chemical barrier. As this layer requires a special type of fixation and methods of investigation it has hitherto escaped investigation. It has strong bactericidal properties. This is due to a hydrogen peroxide producing peroxidase (Kudo and Inoue, 1986, 1989; Kudo *et al.*, 1988).

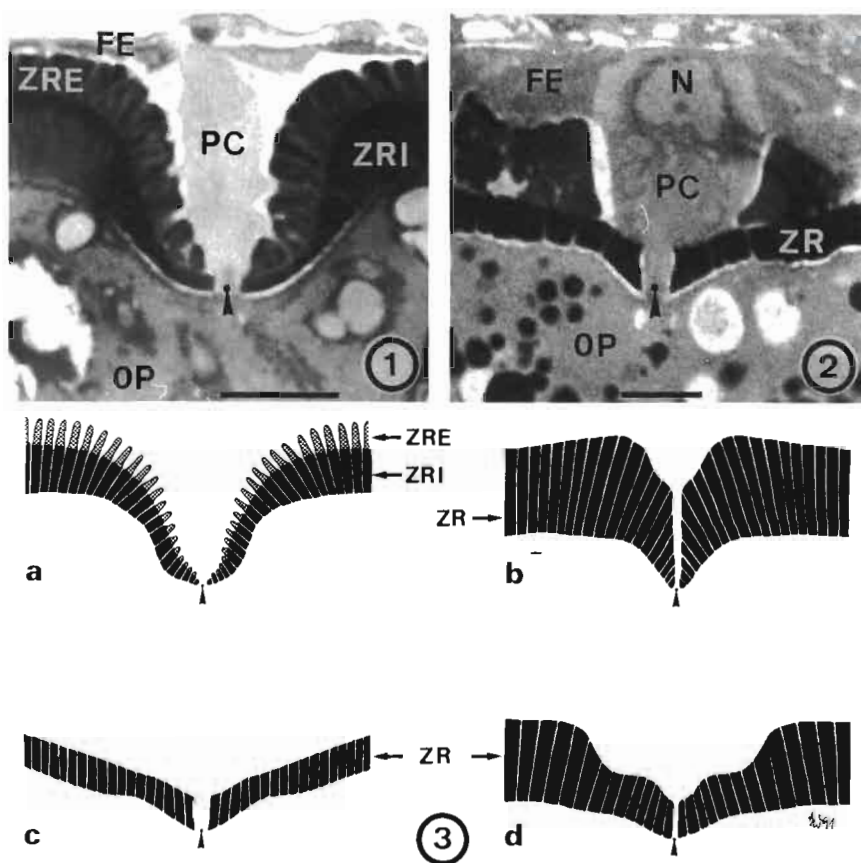
## MICROPYLE

The tough and thick zona radiata represents an impenetrable barrier for sperm cells. In addition, apart from two known exceptions in *Lepidogalaxias salamandroides* (Lepidogalaxiidae; Leung, 1988) and *Gambusia affinis* (Poeciliidae; Baccetti *et al.*, 1988), none of the teleost spermatozoa have acrosomes (Billard, 1970; Mattei, 1970; Baccetti, 1985). Because these vacuoles in the sperm heads contain substances that lyse the various membranous egg envelopes they aid penetration of the genetic material. For this reason, another mechanism for the entry of the sperm into the egg has to evolve to ensure fertilization. This is achieved by a preformed perforation in the envelope, the micropyle.

### Morphological aspects

All teleost fishes here to for investigated only have one micropyle (Cherr and Clark, 1982; Hart, 1990; Depêche and Billard, 1994; compare also Guraya, 1986) but in sturgeons, for example, there may be as many as 52 of these pores (Ginsburg, 1972; Cherr and Clark, 1982).

Most of the teleost micropyles originate in late oocyte-stage I (stages after Arndt, 1956) and their formation is finished in late stage III (Riehl, 1977b; compare also Guraya, 1986; Hart, 1990). The micropyle is normally formed by a particularly large cell of the follicular epithelium that has the shape and function of a plug ("Zapfenzelle") (Figs 1, 2). A cellular extension is shaped very much like the wooden block that is used



Figs 1-3. - FE: follicle epithelium; N: nucleus of plug cell; OP: oocytoplasm; PC: plug cell; ZR: zona radiata; ZRE: zona radiata externa; ZRI: zona radiata interna. The arrowheads point to the micropyle canal.

Fig. 1. - Full-developed micropyle of *Barbatula barbatula* (Balitoridae) with plug cell. It has a deep pit and a short canal (type 1). The arrowhead points to the micropyle canal and the process of the plug cell. (Light microscopy, semithin section, 0.5  $\mu$ m, toluidin blue). Bar = 5  $\mu$ m. Fig. 2. - Full-developed micropyle of *Pomatoschistus minutus* (Gobiidae) with plug cell. This micropyle has no pit (type 3). The micropyle canal is blocked by a process of the plug cell ("Zapfenzelle"). The arrowhead points to the micropyle canal and the process of the plug cell. (Light microscopy, semithin section, 0.5  $\mu$ m, toluidin blue). Bar = 5  $\mu$ m. Fig. 3. - Types of micropyles: (a) type I = micropyles with a deep micropyle pit and a short canal; (b) type II = micropyles with a flat micropyle pit and a correspondingly longer micropyle canal. The micropyle pit is as deep as the micropyle canal is long; (c) type III = micropyles without a micropyle pit with a micropyle canal only. The micropyle may be widened at the upper end; (d) type IV = micropyles with two micropyle pits (outer and inner) and a short micropyle canal.

to close the decanting holes in a barrel (Riehl, 1977b; Brummett and Dumont, 1979; Kobayashi and Yamamoto, 1981, 1985; Ohta and Teranishi, 1982; Takano and Ohta, 1982; Hart and Donovan, 1983; Nakashima and Iwamatsu, 1989). Many microtubules of about 10 nm diameter are arranged in parallel with the long axis of this process

(Kobayashi and Yamamoto, 1985). The plug cell adheres to the zona radiata by means of microvilli.

To date four different types of micropyles have been described (Riehl, 1980; Riehl and Kock, 1989) (Fig. 3):

Type 1: Micropyles with a deep micropyle pit and a short canal (Fig. 4).

Type 2: Micropyles with a flat micropyle pit and a correspondingly longer micropyle canal. The micropyle pit is as deep as the micropyle canal is long (Fig. 5).

Type 3: Micropyles without a micropyle pit but with the micropyle canal only. The micropyle may be widened at the upper end (Fig. 6).

Type 4: Micropyles with two micropyle pits (outer and inner) and a short micropyle canal (Fig. 7).

To prevent the collapse of the micropyle canals, their walls are reinforced by strong annular thickenings as shown on figure 8 (Riehl, 1980; Iwamatsu *et al.*, 1993).

### Functional aspects

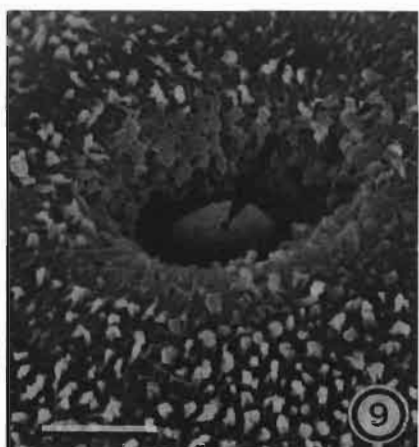
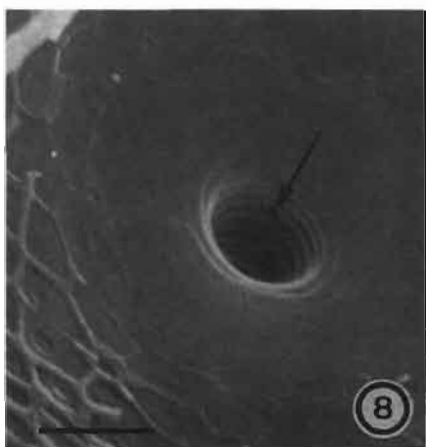
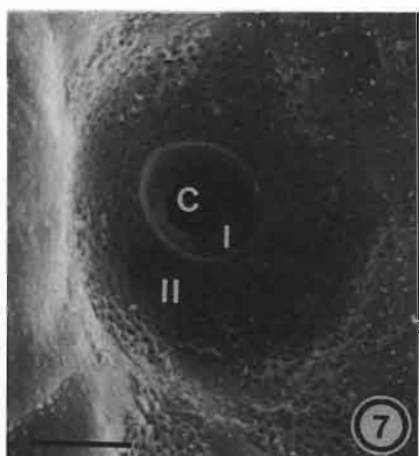
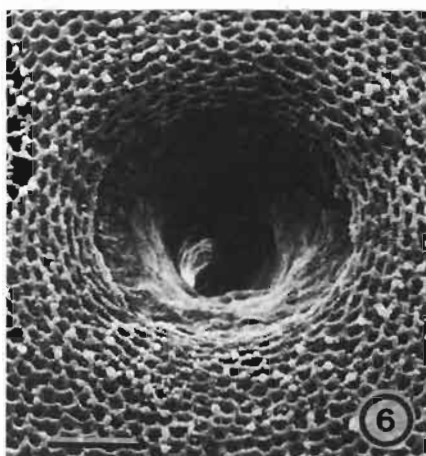
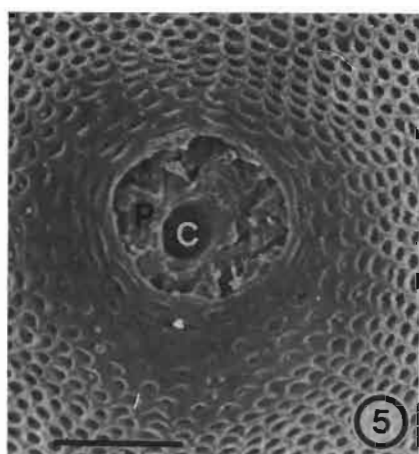
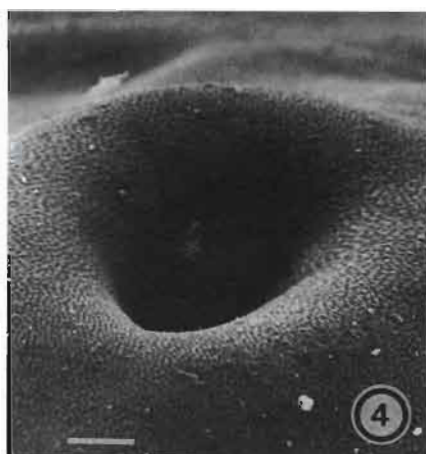
The main purposes of the micropyle are to aid spermatozoa entry through the envelope and to prevent polyspermy. The width of the micropyle canal and the diameter of the spermatozoa head are adapted to one another. The pores are always just wide enough to permit entry of a single spermatozoa. This prevents penetration by more than one spermatozoa and is important because polyspermy leads to abnormal development in teleosts (Ginsburg, 1961, 1972; Kobayashi and Yamamoto, 1981; Jaffe and Gould, 1985; Kobayashi and Yamamoto, 1987; Hart, 1990). Other functions of the micropyle are the attraction of spermatozoa by pheromones (Iwamatsu *et al.*, 1992; Yanagimachi *et al.*, 1992; Iwamatsu *et al.*, 1993) and the influx of water during the formation of the perivitelline space (Kupffer, 1878).

It has been shown recently in the medaka (*Oryzias latipes*, Oryziidae) (Iwamatsu *et al.*, 1991) and in the Schneider (*Alburnoides bipunctatus*, Cyprinidae) (Patzner, Glechner & Riehl, unpubl. data; Riehl, 1996) that the micropyle is closed by a plug of cytoplasm immediately after the first spermatozoa has passed through it (Fig. 9).

Now the question arises: How are the spermatozoa able to find the micropyle? Four different ways are known, namely:

1) By accident. This is the easiest way, because there are immense quantities of spermatozoa; 2) by attraction of pheromones as shown in *Oncorhynchus mykiss*, *O. kisutch*, *Clupea pallasii* and *Oryzias latipes* (Iwamatsu *et al.*, 1992; Yanagimachi *et al.*, 1992; Iwamatsu *et al.*, 1993); 3) by producing sperm trails as found recently in gobiids and blenniids. In these teleosts the male selects a spawning site, e.g. a stone, and deposits spermatozoa on this substrate that live for several hours. Then, the female lays her eggs on these sperm trails (Horsthemke, 1995); and 4. by sperm guiding systems as described recently in cyprinids (Amanze and Iyengar, 1990; Riehl and Patzner, 1994), loricariids (Riehl and Patzner, 1991) and anabantoids (Riehl and Kokoscha, 1993; Britz *et al.*, 1995). Amanze and Iyengar (1990) showed by time-lapse video microscopy and computer-aided analysis of sperm motility pattern in the micropylar region that the fertilizing sperm always travelled preferentially along the grooves into the micropylar pit. The sperm guidance role of the micropylar region was calculated to enhance chances of egg penetration/fertilization by as much as 99.7% once sperm were within the micropylar region.







In loricariid *Sturisoma* low magnification of the outer surface of the eggs reveals a pattern that is unique among all teleost fishes examined to date. All eggs studied had 22 furrows that ran from the animal to the vegetative pole (Fig. 10). The micropyle consists of a canal that widened outward like a funnel. The area that surrounded the micropyle canal exhibited no special pattern, except for the furrows that terminated near the micropyle canal (Fig. 11). The wall of the canal was reinforced by strong annular thickenings (Riehl and Patzner, 1991).

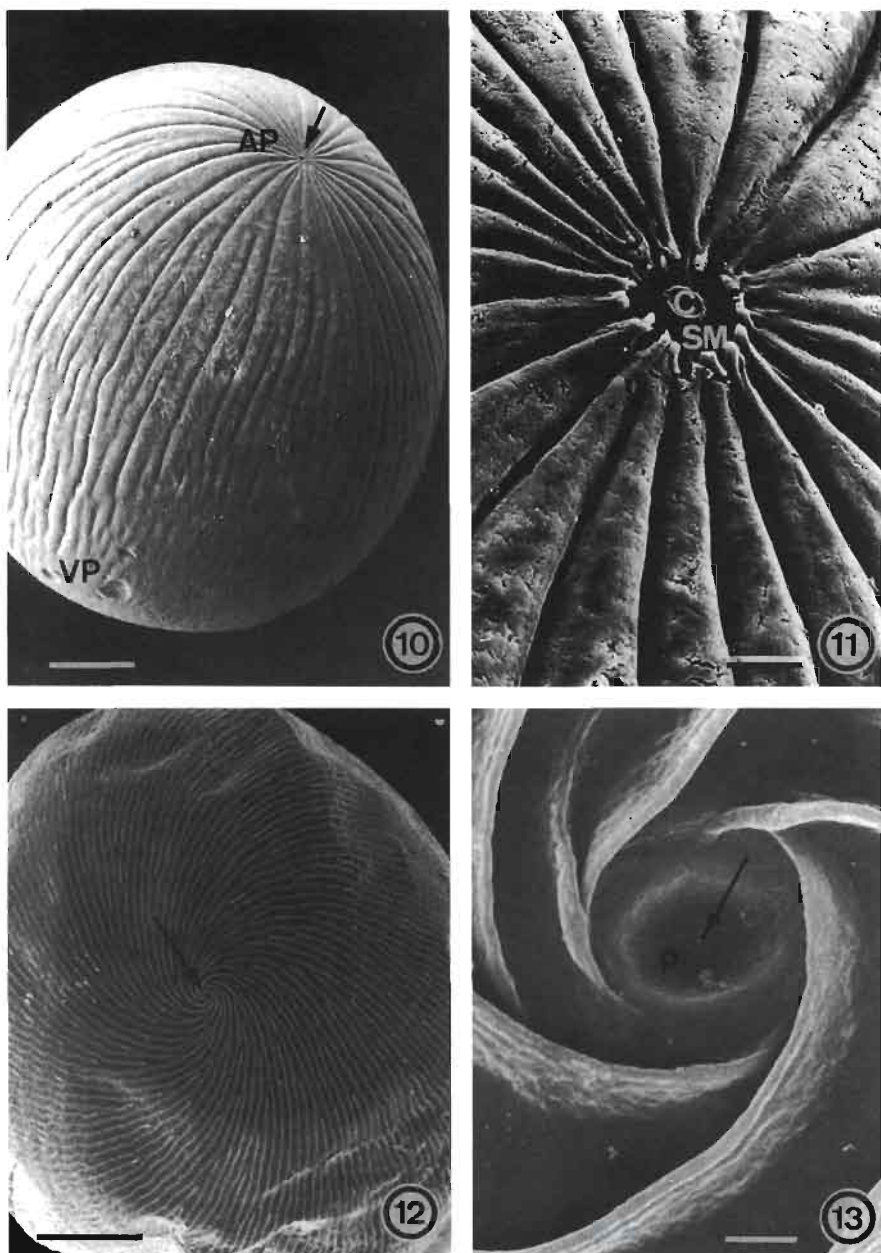
The outer surface of the zona radiata in the perciform *Luciocephalus* reveals a pattern that is different at the vegetal and animal poles. Originating from an irregular pattern at the vegetal pole, the pole opposite the micropyle, numerous spiralling ridges extend parallel in the micropyle (Fig. 12). As the diameter of the egg increases from pole to the equator, the number of ridges also increases, and is highest in the equatorial region of the egg. Most ridges stop at various distances from the micropyle. Only some of them actually reach the micropylar region and end at the edge of the micropylar pit in a counter-clockwise directed spiral (Fig. 13). This spiralling pattern is also found in anabantoids (Riehl and Kokoscha, 1993; Britz *et al.*, 1995).

**Acknowledgements.** - I thank Mrs. Gisela Reuter (Düsseldorf) and Mag. Regina Glechner (Salzburg) for their help with the preparation of the micrographs and Dr. Volker Walldorf (Düsseldorf) for drawing figure 3.

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Figs 4-9 - C: micropyle canal; P: micropyle pit.

Fig. 4. - Micropyle of *Chalcalburnus chalcoides mento* (Cyprinidae). This micropyle belongs to type 1. Bar = 10 µm. Fig. 5. - Micropyle in an egg of *Gymnocephalus cernuus* (Percidae). The micropyle consists of a shallow pit (P) and a long canal (C) (type 2). Bar = 10 µm. Fig. 6. - The micropyle of *Coregonus nasus* (Coregonidae) belongs to type 3. Bar = 5 µm. Fig. 7. - Micropyle of *Lepidonotothen larseni* (Nototheniidae) consisting of two pits (I + II) (rare type 4). Bar = 10 µm. Fig. 8. - Micropyle canal of *Champocephalus gunnari* (Channichthyidae) with strong annular reinforcements (arrow). Bar = 5 µm. Fig. 9. - Micropyle of the cyprinid *Alburnoides bipunctatus* (Cyprinidae) showing a cytoplasmatic plug (arrow) which closes it immediately after the first spermatozoon has passed (Photo: Regina Glechner). Bar = 10 µm.



Figs 10-13. - AP: animal pole; M: micropyle; P: micropyle pit; SM: smooth region; VP: vegetal pole.

Fig. 10. - Total view of an egg of *Sturisoma aureum* (Loricariidae). Note the deep furrows which run from the animal to the vegetal pole. The arrow points to the micropyle. Bar = 250  $\mu$ m. Fig. 11. - Micropyle of *S. aureum*. The micropyle is surrounded by a smooth region. Bar = 10  $\mu$ m. Fig. 12. - Low power micrograph of a *Luciocephalus* sp. egg (Luciocephalidae). The arrow points to the micropyle. Bar = 500  $\mu$ m. Fig. 13. - The micropyle of *Luciocephalus* sp. consisting of a micropyle pit (P) and a micropyle canal (arrow) in the centre. Bar = 10  $\mu$ m.

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## Workshop 1

### PARASITOLOGY AND ICHTHYOLOGY

Chairpersons: **Louis EUZET & Elisabeth FALIEUX**

- FALIEUX E. - What can parasite studies provide to Ichthyology ? 603-615  
*Qu'apporte l'étude des parasites à l'Ichtyologie ?*
- NAHHAS F.M. & A. GREWAL. - Digenetic Trematodes from marine fishes of Fiji: subfamily Hurleytrematinae (family Monorchhiidae): A review and description of four new species of *Hurleytrematoides*. 617-631  
*Revue et description de quatre nouvelles espèces de Hurleytrematoides (Hurleytrematinae, Monorchhiidae): Trématodes digènes parasites de poissons marins de Fiji.*
- RIGBY M.C. - The Camallanidae (Nematoda) of Indo-Pacific fishes: Taxonomy, ecology and host-parasite coevolution. 633-644  
*Les Camallanidae (Nematoda) des poissons indo-pacifiques: taxinomie, écologie et co-évolution hôte-parasite.*





## WHAT CAN PARASITE STUDIES PROVIDE TO ICHTHYOLOGY ?

by

Elisabeth FALIEUX (1)

**ABSTRACT.** - The aim of this review is to emphasise the value of parasitological studies as a tool for better understanding various aspects of fish biology. Numerous studies of fish parasites have proven to be extremely useful in providing informations on, for example, fish diet and feeding behaviour, spawning behaviour, recruitment pattern of juveniles, adult migration, stock and population separation, taxonomy and systematics, phylogeny, and center of origin and ancestral dispersal routes. The usefulness of parasitology in studying fish biology is presented in this short review using specific examples from the literature.

**RÉSUMÉ.** - Qu'apporte l'étude des parasites à l'ichthyologie ?

Le but de cette courte revue bibliographique est de mettre en évidence l'intérêt de l'approche parasitologique comme outil apportant des informations complémentaires sur divers aspects de la biologie des poissons. En effet, de nombreux travaux montrent l'intérêt de l'utilisation des parasites de poissons comme marqueurs biologiques dans des domaines variés de l'ichthyologie tels que, par exemple: comportement nutritionnel et régime alimentaire, comportement de ponte, recrutement de juvéniles, migrations, identification ou discrimination de stock et/ou de population, taxonomie et systématique, phylogénie, centre d'origine géographique et colonisations ancestrales. Dans cette brève revue, l'utilité de la parasitologie pour l'étude de la biologie des poissons est illustrée à l'aide d'exemples empruntés à la littérature.

**Key-words.** - Ichthyology, Parasitology, Review.

The aim of this review is to emphasise the value of parasites as natural tags for better understanding fish biology. The utility of parasitological approaches in fish biology is known since 1939 with the first studies of Herrington *et al.* who worked on population of redbfish, *Sebastes marinus* in the Gulf of Maine, and of Dogiel and Bychowsky who distinguished between two groups of acipenserids in the Caspian Sea and later with the pioneering studies of Manter (1955), Szidat (1955), Margolis (1956, 1963, 1965), Kabata (1959, 1963), Sinderman (1961), and others. Now, with the increase of knowledge on parasite fauna of fishes and on parasites themselves, specially life cycle, life span, ..., they are numerous papers that shown the successful use of parasites in providing informations on various aspects of fish biology such as: fish diet, migrations of fish, stock and population separation, taxonomy and systematics, phylogeny, center of origin of fish and dispersal routes.

These authors also highlight the advantages of using parasites as biological tags (Table I).

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Potentially, all taxonomic groups of parasites (from fungi to crustacea) can be used as natural tags. However, the selection of suitable parasite tags for various studies of fish biology must follow some general criteria (Table II).

MacKenzie (1983) gives, in a summary form, a useful list of the parasite groups considered most likely to provide biological tags appropriate to different types of fish population study and of subject host (Table III).

Depending on the type of study certain criteria for selecting a suitable parasite as a biological tag are more important than others. For ichthyological studies of taxonomy and systematics, phylogeny, center of origin and ancestral dispersal routes, and evolutionary biology of fish it is necessary to use parasite taxa which are highly specific to their host species. The concept of host specificity has been defined by Lambert and El Gharbi (1995) as « the way of characterizing the relationships that exist between the

Table I. - The advantages of using parasites as tags (taken from Kabata, 1963; MacKenzie, 1983; Sindermann, 1983; Lester, 1990; Moser, 1991; Williams *et al.*, 1992).

Natural tagging by parasites	
1	Requires a smaller number of fish collected than does for artificial tagging (means mechanical), since fish need only to be caught once.
2	Is less expensive than artificial tagging.
3	Requires no reliance on the general public for tag return.
4	Reflects the entire fish population whereas artificial tags mark only individuals within a population.
5	Is based on the recording of natural features, so natural tagging eliminates doubts concerning the possible abnormal behaviour or mortality of artificially tagged fish (of course parasites selected as suitable tags are those that do not cause selective mortality or behavioural changes of their hosts).
6	Can be combined with biochemical, serological, morphometric-meristic studies of the same sample.
7	Can be enhanced further by work on parasite biochemical speciation or strain differentiation.
8	Moreover, in some cases natural tagging by parasites is more appropriate than artificial tagging, specially for deep sea fish (such as redfish of the genus <i>Sebastes</i> ) or fragile fish (as clupeids for example) because of their high mortality after trawling.

Table II. - Criteria for the use of parasites as tags (taken from Sindermann, 1961, 1983; Kabata, 1963; MacKenzie, 1983; Lester, 1990; Moser, 1991; Williams *et al.*, 1992).

1	Differences in prevalence and / or in abundance should exist in the different parts of the studies area.
2	Prevalence should be stable from season to season and from year to year but seasonal variations can determine seasonal migrations of fish.
3	Parasites' life span or remaining of parasites in an identifiable form should be long enough to cover the time scale of investigation. Lester (1990) considers this criterion to be the most important one for judging the suitability of a parasite as tag.
4	Parasite should be detected easily, preferably by gross examination.
5	Life cycle of the parasite selected should preferably involve a single host species but some authors (Lester, 1990) consider now that this criterion is incorrect. This restricting choice would eliminate many potentially useful tags.
6	Definitive identification of the parasite should be feasible but is not essential. One needs only to be able to separate the types of parasite.
7	Parasite should have no effect on survival and behaviour of the host.
8	A good knowledge of the range of tolerance of the different stages of a parasite tag, and of its hosts to various environmental factors is important.

host and parasite and which confront their two genomes in a given environment ». For parasites, to be « highly specific » to their host species means that they can just infest a few closely related host species, for example species of the same genus, - defined as « narrow specificity » - or even only a single host species - « strict specificity » - (Euzet and Combes, 1980).

It is therefore important to find the appropriate parasite taxa that show high specificity to their host species. Sasal *et al.* (in press) have observed, for example, that parasites characterized by a simple or direct life cycle (only one individual host is needed to achieve the life cycle) may have a higher specificity to their fish host than other parasite taxa with a complex or indirect life cycle (one definitive host and intermediate hosts in which respectively adult stage and larval stages of the parasite develop successively in order to achieve the life cycle is needed). This has been reported for Monogenea and Copepoda, which have a simple life cycle in contrast to Nematoda and Acanthocephala, which have a complex life cycle (Sasal *et al.*, in press). One exception to this general pattern is the case of Eucestoda which, although having complex life cycle, shows a narrow range of host species (Sasal *et al.*, in press).

The usefulness of parasites as tags has been proven in studying various aspects of fish biology.

### Fish diet and feeding behaviour of fish

References: e.g., Margolis, 1965; MacKenzie, 1983; Lester, 1990; Moser, 1991; Williams *et al.*, 1992.

Usually, the variety of animals used as intermediate hosts for parasites with complex life cycles is more or less restricted. So, the general feature of the food of a given fish can be directly deduced from the endoparasites it harbours. Moreover, the parasites persist for a relatively long time in the gut of their fish host whereas food is rapidly digested.

Table III. - A list of parasite groups considered most likely to provide biological tags appropriate to different types of fish population study and different types of subject host (taken from MacKenzie, 1983). Key to parasite groups: Aa, acanthocephalan adults; Al, acanthocephalan larvae; Ca, cestode adults; Cl, cestode larvae; Cr, crustaceans; Da, digenean adults; Dm, digenean metacercariae; Mo, monogeneans; My, myxosporeans; Na, nematode adults; Nl, nematode larvae; Pr, protozoans; Tl, trypa-norhynch cestode larvae.

	Types of subject host								
	Pelagic marine teleosts		Demersal marine teleosts		Anadromous teleosts		Sharks, skates and rays		
Types of fish population study	Planktophagous species	Piscivorous species	Benthophagous species	Piscivorous species	Salmonids		Sturgeons	Ca, Cr, Na, NI	
Stock separation	Dm, NI, Pr, TI	Aa, Al, Cr, Mo, NI, TI	Al, Cl, Dm, Na, NI, Pr	Aa, Cl, Cr, Mo, My, NI	Freshwater parasites	Marine parasites	Mo, Na, NI		
Recruitment studies	Dm, TI	Al, TI	Al, Dm	TI	Cl, My, Na	–	Na, NI		NI
Seasonal migrations	Da	Aa, Da	Aa, Da, Na	Aa, Ca, Da	Ca, Da	Aa, Da	Aa, Da		Ca
Adult age-dependent migrations	TI	Cr, TI	Al, Cl, Dm	Cr, TI	Cl, My	Aa, Da, NI	Na, NI		NI

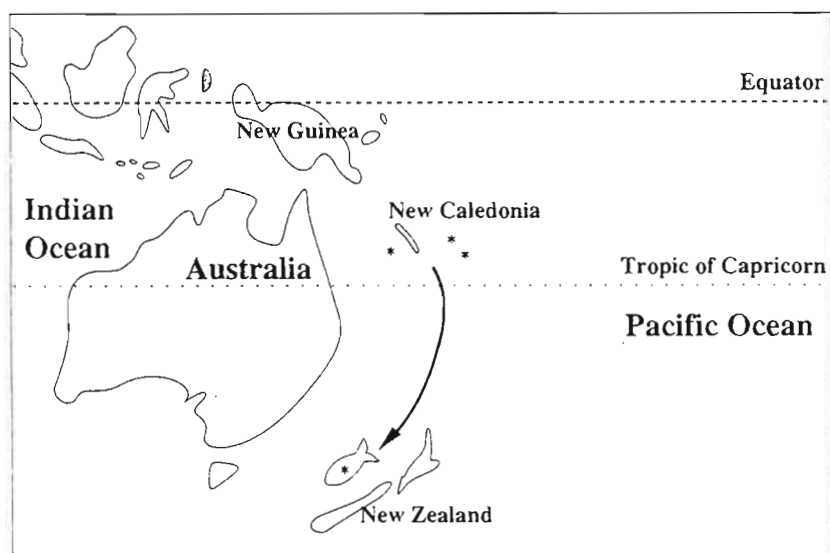


Fig. 1. - Migration of Skipjack tuna, *Katsuwonus pelamis* of New Zealand (adapted from the work of Lester *et al.*, 1985; Lester, 1990). \*: didymozoid digeneans.

According to Margolis (1965), sockeye salmon (*Oncorhynchus nerka*) from North Western Pacific contained more *Echinorhynchus gadi*, an acanthocephalan, than did those from North Eastern Pacific. As this parasite used an amphipod as intermediate host and was widely distributed in other fish species, Margolis concluded that the diet of sockeye salmon from North Western Pacific probably contained more amphipods than did the diet of sockeye salmon from North Eastern Pacific.

Table IV. - Average number of didymozoids in skipjack tuna, *Katsuwonus pelamis*, caught around New Zealand compared with the average number of the same parasites collected in skipjack from tropical areas (taken from Lester *et al.*, 1985). A: Stomach; B: Intestine.

Parasite	New Zealand	Tropics
<i>Didymocylictricus filiformis</i>	9	6
<i>D. simplex</i>	20	12
<i>Didymoprolema fudiforme</i>	2.7	2.5
<i>Lobatozoum multisacculatum</i>	0.3	0.1
<i>Coeliodidymocystis</i> sp.	1.1	0.9
<i>Oesophagocystis dissimilis</i>	7	9
<i>Kollikeria/Didymocystis</i> spp.	5	6
<i>Didymocestoides intestinomuscularis</i> <sup>A</sup>	15	41
<i>D. intestinomuscularis</i> <sup>B</sup>	6	7
<i>Lagenocystis katsuwoni</i> / <i>Univitellannulocystis katsuwoni</i>	26	47
Number of fish	232	469

### Recruitment pattern of juveniles, adult migrations and spawning migrations of fish

References: e.g., Sindermann, 1961, 1983; Margolis, 1963, 1965; Black, 1981; Dick and Belosevic, 1981; Frechet *et al.*, 1983; Frimeth, 1987; Groot *et al.*, 1989; Moser, 1991; Williams *et al.*, 1992; Speare, 1994, 1995; Konovalov, 1995; MacKenzie and Longshaw, 1995.

The parasite fauna of a given fish reflects the habitat previously occupied by this fish. Then, subsequent movements of a fish can be deduced if we know where this fish has acquired a parasite.

One example is the one of skipjack tuna, *Katsuwonus pelamis*, of New Zealand waters (from the works of Lester *et al.*, 1985; Lester, 1990) (Fig. 1, Table IV). The gills of skipjack tuna caught around New Zealand harbour 9 species of didymozoids (digeneans). However, didymozoids are almost entirely tropical or subtropical parasites. They are largely unknown from fishes that are endemic of temperate waters such as those around South Australia and New Zealand. In this case, didymozoids collected in fish from New Zealand are similar in number than those for fish taken in adjacent tropical areas. From these data, authors concluded that skipjack tuna caught in New Zealand waters spent the previous few months in the tropic and subtropics and are not recruited from juveniles living in New Zealand waters.

### Stock and population separation

References: e.g., Margolis, 1956, 1990; Kabata, 1959, 1963; Arthur and Arai, 1980; Sindermann, 1983; Rohde, 1987; Kabata *et al.*, 1988; Lester *et al.*, 1988; Lester, 1990; Hemmingsen *et al.*, 1991; Moser, 1991; Stanley *et al.*, 1992; Arthur and Albert, 1993; Khan and Tuck, 1995; Konovalov, 1995; Sewell and Lester, 1995; Speare, 1995; Boje *et al.*, 1997.

The analysis of the number of parasites in individual fish from different geographical areas can provide informations on host movements and thus, can provide evidence of isolated or semi-isolated populations.

For example, Rohde (1987) was able to differentiate populations of *Scomber australasicus* from New Zealand and from South East of Australia New South Wales only using sclerite size, and specially the length of hamuli, of a monogenean, *Kuhnia scomбри* (Fig. 2, Table V). From the data obtained in his study, this author showed that there are significant differences between populations of *Kuhnia scomбри* from *Scomber australasicus* in New Zealand and in New South Wales. Since Rohde and Watson (1985a, 1985b) have shown that length of sclerites in *Kuhnia* does not change with the size of worms, Rohde argued that monogenean sclerites appear to be well suited for differentiating and comparing different fish populations. Finally, this author concluded that a simple method, easy to employ and not very time consuming, can be very useful for comparing different fish populations.

Table V. - *Kuhnia scomбри*. Length distribution of hamuli in two populations of *Scomber* in the Pacific. t-test showed significant differences ( $P < 0.001$ ) between the two localities.

Locality and host	Length ( $\mu\text{m}$ )							
	96-100	101-105	106-110	111-115	116-120	121-125	126-130	131-135
New South Wales <i>Scomber australasicus</i>	3	12	16	2				
New Zealand <i>S. australasicus</i>			1	16	39	54	9	1

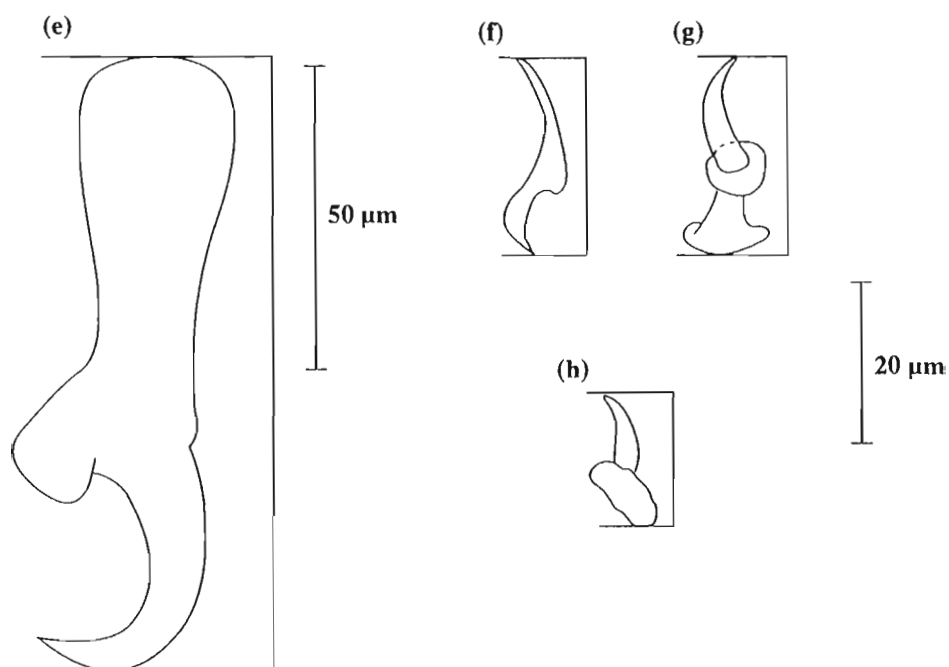


Fig. 2. - Population separation of *Scomber australasicus* from New Zealand and South East of Australia (New South Wales) (Adapted from Rohde, 1987). Sclerites of *Kuhnia scombri*: (e) hamulus; (f) marginal I; (g) large genital hook; (h) small genital hook. Lines along sclerites indicate how measurements were taken.

### Taxonomy and systematics of fish

References: e.g., Rokicki, 1983; Lambert and Romand, 1984; Cloutman, 1988; Euzet *et al.*, 1989; Paugy *et al.*, 1990; Lambert and El Gharbi, 1995.

The work of Euzet *et al.* (1989) gives a good example of the usefulness of parasites for fish systematics (Table VI). In this work, two independent studies were conducted on 40 siluriform fish belonging to the genus *Chrysichthys* from Ivory Coast. Identification of fish species of this genus is difficult and uncertain on morphological characters alone.

The first study, using enzyme polymorphism, revealed 3 groups of fish corresponding to 3 distinct species which belong to *C. auratus* (22 individuals), *C. maurus* (2 individuals) and *C. nigrodigitatus* (16 individuals). The second study, using identification of gill monogeneans, was undertaken on the same sample of fish. Six species of monogeneans, all belonging to the genus *Protoanclodiscoïdes* were collected. Correspondence analysis, based on parasite data, revealed the existence of 3 groups of host. The comparison of the genetic characteristics and the monogenean species of each fish showed a close correlation. These authors concluded that monogeneans were here as good indicators of their hosts as were the enzymes.

As Lambert and El Gharbi (1995) said: « certain parasites were shown to be good fish systematists ».

Table VI. - Usefulness of parasites for siluriform fish (genus *Chrysichthys*) systematics (taken from Euzet *et al.*, 1989). From 1 to 40: Host samplings (N° 33 and 35 free of parasites); list of the *Protoancylo-discoides* species; the A, B, C groups of hosts according to the genetic data (respectively *C. auratus*, *C. maurus*, and *C. nigrodigitatus*).

Samples	Parasites						Hosts
	<i>P. chrysichthes</i>	<i>P. auratum</i>	<i>P. combesi</i>	<i>P. sp. 1</i>	<i>P. sp. 2</i>	<i>P. sp. 3</i>	
1		33	37	3			A
2	3	25	9	3			A
3				41	41		B
4		8	8	1			A
5	5	22	29	24			A
6	2	44	10	2			A
7	5	27	25	1			A
8		39	16	2			A
9		10	16	4			A
10		1		36	23		B
11	2	46	14	4			A
12	5	19	11	3			A
13		7	5				A
14		12	23	4			A
15	28		2	6			C
16		1	1	1			A
17		30	24				A
18		9	6				A
19	1	23	18	1			A
20	1	7	7	1			A
21		3	3				A
22		16	19				A
23		18	5				A
24		16	16	3			A
25		1	12				A
26	16		1		2	26	C
27	17		3		2	19	C
28	25		4	3	1	10	C
29	11		3	8		16	C
30	25		2			20	C
31	5		1	5		9	C
32	25		1	1		10	C
34	13		8			9	C
36	11		2		1	5	C
37	15		2		1	12	C
38	6		1				C
39	4		8			2	C
40	3		1			1	C

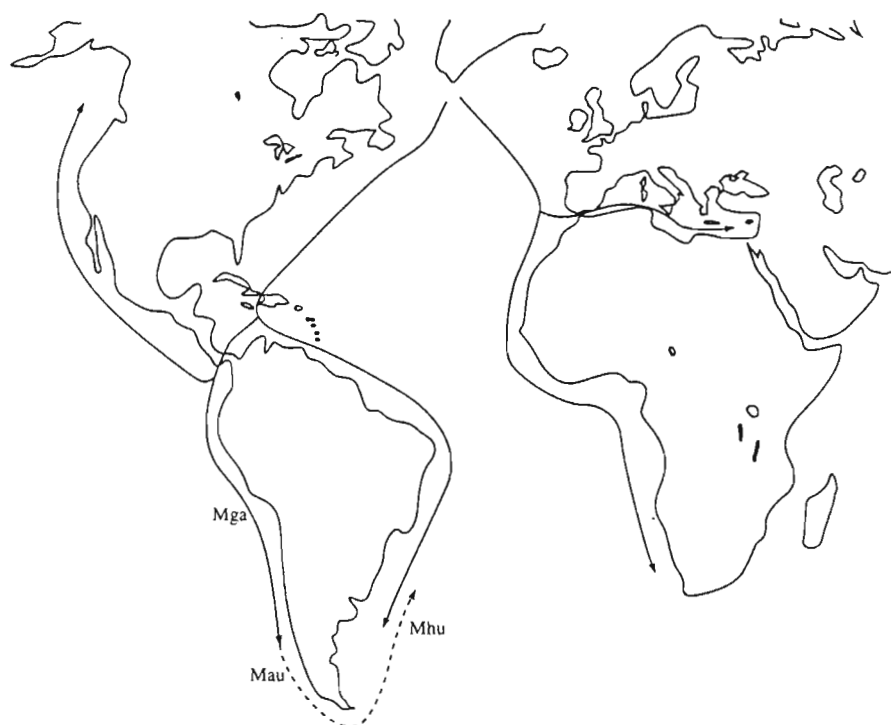


Fig. 3. - Origin and dispersal of the genus *Merluccius* according to the hypotheses of Kabata and Ho (1981) and Inada (1981). The only disagreement between hypotheses of Inada and Kabata and Ho resides in the origin and dispersal of *M. hubbsi* and *M. australis*. Continuous lines indicate dispersal of these two species according to Kabata and Ho (1981) and broken lines according to Inada (1981) (taken and adapted from Esch and Fernandez, 1993). Mau: *M. australis*; Mga: *M. gayi*; Mhu: *M. hubbsi*.

### Phylogeny of fish

References: e.g., Klassen and Beverley-Burton, 1987; Williams *et al.*, 1992; Ti-rard *et al.*, 1992, 1993; Orti *et al.*, 1996; Thomas *et al.*, 1996.

Because some parasites have evolved coincidentally with their hosts, studies of parasites can provide clues to host phylogeny.

For example, Klassen and Beverley-Burton (1987) compared the phylogenetic relationships of the ancyrocephalid monogeneans, *Ligictaluridus* spp. (based on a cladistic analysis involving the morphology of the male copulatory apparatus) with two phylogenies of their ictalurid hosts previously proposed by Taylor (1969) and Lundberg (1970). Even if the parasite cladogram of Klassen and Beverley-Burton (1987) did not perfectly match either of the host cladograms, Lundberg's host phylogeny was supported by the phylogeny of the parasite. Although host and parasite phylogenies did not provide a perfect match, mismatches could be explained by ecological events such as host switching due to for example changes in the environment. Ecological factors have to be taken into account when comparing host and parasite phylogenies, but nevertheless parasite phylogenies can provide additional information to resolve the phylogenies of their hosts.



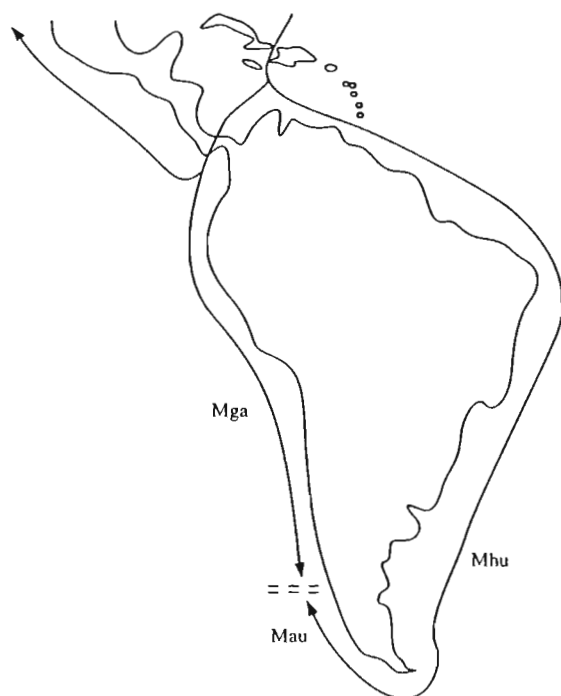


Fig. 4. - Possible dispersal of *Merluccius* around South American continent based on the parasites of *M. australis* from the coast of Chile (taken and adapted from Esch and Fernandez, 1993). Mau: *M. australis*; Mga: *M. gayi*; Mhu: *M. hubbsi*.

#### Center of origin and ancestral dispersal routes

References: e.g., Manter, 1955; Szidat, 1955; Margolis, 1965; Brooks *et al.*, 1981; Kabata and Ho, 1981; Black, 1983; Fernandez, 1985; Fernandez and Duran, 1985; Ibragimov, 1987; Esch and Fernandez, 1993.

It is assumed that hosts have a greater diversity of parasites in areas they have occurred for a long time than in areas they have more recently colonised. Then, the dispersal of hosts into new areas may lead to the loss of their original parasites, or to the acquisition of new ones. Thus, parasites present in the area of origin should be regarded as ancestral to those present in the newly colonised areas.

The fruitful use of parasites in determining center of origin and ancestral dispersal routes has been demonstrated in the case of the fish species belonging to the genus *Merluccius* (Figs 3, 4). There is a long-standing controversy regarding the geographical origin and spreading of the species belonging to this genus. In 1948, Svetovidov hypothesised that the genus *Merluccius* evolved in the present day North Atlantic. On the contrary, Szidat (1955) suggested that *Merluccius* originated in the North Pacific and specially in the sea of Bering. More than 25 years later, Inada (1981) and Kabata and Ho (1981) examined the problem using respectively ichthyological data and parasitological informations (specially specific copepods). These authors independently reached similar conclusions, but opposite to Szidat's. They argued that the genus *Merluccius* arose in the North Atlantic and spread out following 2 routes (Fig. 3):

- One following the Atlantic coast of Europe and Africa, including a group that branched off into the Mediterranean.

- A second one migrating south along American coast and into the Pacific through Isthmus of Panama which was then submerged. Once they reached the Pacific, they followed 2 different routes, one moving northward and the other southward.

The only disagreement between hypotheses of Inada and of Kabata and Ho resides in the origin of *Merluccius hubbsi* and *M. australis*. According to Kabata and Ho, *M. hubbsi* originated from the Atlantic stock that split in the area of Isthmus of Panama and migrated South down. On the contrary, Inada argued that *M. hubbsi* and *M. australis* originated in the Pacific, *M. hubbsi* moving toward the Atlantic around southern end of South America. Also, Kabata and Ho indicated that, from a parasitological point of view, *M. australis* from New Zealand was more closely related to *M. hubbsi* from South Atlantic than to *M. gayi* from the South Pacific.

In 1985, Fernandez, and Fernandez and Duran studied the parasites of Patagonic population of *M. australis* using specific copepods and a sanguinicolid digene (Fig. 4). They concluded that *M. australis* was closely related to *M. hubbsi* and unrelated to *M. gayi*. They supported then Kabata and Ho hypotheses regarding an Atlantic origin for *M. hubbsi* and *M. australis*.

## CONCLUSION

As a conclusion to this short review, we argue that parasite studies can be fruitfully used in various aspects of fish biology as a source of complementary informations, which should be combined with other methods such as molecular markers or more traditional tools. Moreover, parasite tags are of particular value for movement and migration studies of fishes (Lester, 1990). In some cases, natural tagging by parasites may be the most appropriate method for studying some aspects of fish biology. However, for a successful use of parasite as tags a background in parasitology is required, and an extensive preliminary work (which is time-consuming) is often necessary in order to select suitable parasites (and the more types of parasites are selected, the more sensitive is the method) for answering the questions asked.

Otherwise, the author would like to highlight the particular interest of some parasite taxa as valuable biological tags in some aspects of ichthyological studies (taxonomy and systematics, phylogeny, center of origin and ancestral dispersal routes, and evolutionary biology of fish). Parasites with a high specificity to their hosts have been shown to be useful in studies concerning for example the taxonomy and systematics of fish. In case of strict host specificity, Lambert and El Gharbi (1995) said that « parasite presence can be used as diagnostic criterion for the host species, the parasite being sufficient to identify the host with precision ». Another example is the study of fish phylogenies. Cospeciation occurring more frequently with highly specific parasites than with poorly specific parasites (Humphery-Smith, 1989) then, genealogical relationships of parasites can be considered as a source of informations for genealogical relationships of their hosts.

Finally, in their paper dealing with the use of parasites as host evolutionary prints, Thomas *et al.* (1996) pointed out that « Parasitologists should promote this unique attribute of parasites to Systematists and Evolutionists », because parasites are a singular group of organisms which can tell us a lot about their hosts as well.

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**DIGENETIC TREMATODES FROM MARINE FISHES OF FIJI:  
SUBFAMILY HURLEYTREMATINAE  
(FAMILY MONORCHIIDAE): A REVIEW AND DESCRIPTION OF  
FOUR NEW SPECIES OF *HURLEYTREMATOIDES***

by

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**ABSTRACT.** - Four new species of *Hurleytrematoides* Yamaguti, 1954 are described: *H. fijiensis* from *Heniochus acuminatus* and *Chaetodon citrinellus*, *H. prevoti* from *Chaetodon melannotus*; *H. bartolii* from *Heniochus acuminatus* and *H. monoceros*, and *H. zebrasomae* from *Zebrasoma scopas*. The hurleytrematine genera *Hurleytrema*, *Hurleytrematoides*, *Pseudohurleytrema* and *Parahurleytrema* are briefly discussed and their validity accepted based chiefly on the structure of the male and female terminal structures. *Hurleytrema pyriforme* Overstreet, 1969 and *H. catarinensis* Amato, 1982 are transferred to *Pseudohurleytrema* as new combinations. The generic diagnosis of *Hurleytrematoides* is emended to include cirrus with large and extensive spines or small ones with limited distribution, and a spiny or spinose unipartite terminal organ. A key to the hurleytrematine genera with single testis and their species is given.

**RÉSUMÉ.** - Revue et description de quatre nouvelles espèces de *Hurleytrematoides* (Hurleytrematinae, Monorchiidae): Trématodes digènes parasites de poissons marins de Fidji.

Quatre nouvelles espèces de *Hurleytrematoides* Yamaguti, 1954 sont décrites: *H. fijiensis* de *Heniochus acuminatus* et *Chaetodon citrinellus*, *H. prevoti* de *Chaetodon melannotus*, *H. bartolii* de *Heniochus acuminatus* et *Heniochus monoceros*, et *H. zebrasomae* de *Zebrasoma scopas*. Les genres de la sous-famille des Hurleytrematinae, *Hurleytrema*, *Hurleytrematoides*, *Pseudohurleytrema* et *Parahurleytrema*, sont brièvement traités. Leur validité est confirmée par les structures terminales des mâles et femelles. *Hurleytrema pyriforme* Overstreet, 1969 et *H. catarinensis* Amato, 1982 sont transférés dans le genre *Pseudohurleytrema*. *Hurleytrematoides* se caractérise principalement par la présence de cirres ayant des épines larges et étendues ou petites et à distribution limitée, et par un organe terminal épineux ou pas. Une clé de la sous-famille est proposée.

**Key-words.** - Fish Parasites, Digenetic Trematodes, Monorchiidae, Hurleytrematinae, *Hurleytrematoides fijiensis*, *H. prevoti*, *H. bartolii*, *H. zebrasomae*, ISEW, Fiji, New species.

The present paper is based on a collection of digenetic trematodes obtained by the senior author between 13 January and 7 February 1992, at Suva, Fiji. Prior to this collection, two others had been made: the first by Manter in 1951 (see Manter, 1953, 1961, 1963a, 1963b, 1963c) and the second between 1979 and 1982 by the *Hatsutori Maru* and other fishing boats on charter to the government of New Zealand (see Lester *et al.*, 1985). Manter (1963c) reported one monorchiid, *Proctotrema himezi* (Yamaguti, 1951) Manter and Pritchard, 1961 from *Parupeneus indicus*; Lester *et al.* (1985) reported none. The pres-

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ent paper describes four species of hurleytrematine monorchiids and is the third on digeneans. The two previous reports dealt with the family Gyliuchenidae Ozaki, 1933 (Nahhas and Wetzel, 1995) and the family Haplospilichnidae Poche, 1926 (Nahhas *et al.*, 1997).

## HISTORICAL REVIEW

The family Monorchiidae was named by Odhner in 1911 to accommodate *Monorchis monorchis* (Stossich, 1890) Looss, 1902 from the teleosts *Cantharus orbicularis* and *Oblada melanura*. To date the family includes at least 290 nominal species in 58 genera. Three major reports dealing with classification of monorchiids into subfamilies are those of Manter and Pritchard (1961), Mamaev (1968) and Yamaguti (1971). Manter and Pritchard placed all species reported to that date in five subfamilies: Monorchiinae, Asymphyliodorinae, Hurleytrematinae, Opisthomonorchinae, and Octotestiinae; Mamaev recognized the same five subfamilies. Yamaguti placed all monorchiids in 12 subfamilies: Monorchiinae, Anamonorchinae, Ancylocoeliinae, Asymphyliodorinae, Hurleytrematinae, Lasiotocinae, Opisthomonorchinae, Opisthomonorchinae, Postmonorchinae, Pseudopalaeorchiinae, Pseudoproctotrematinae and Telolecithinae.

Among the 58 monorchiid genera, five are characterized by a single testis and eggs with filaments: *Hurleytrema* Srivastava, 1939, *Opisthomonorchis* Yamaguti, 1952, *Pseudohurleytrema* Yamaguti, 1954, *Hurleytrematoides* Yamaguti, 1954, and *Parahurleytrema* Nahhas & Powell, 1965. The genital pore in *Opisthomonorchis*, (subfamily Opisthomonorchinae) is post-acetabular; in the others (subfamily Hurleytrematinae) pre- or par-acetabular.

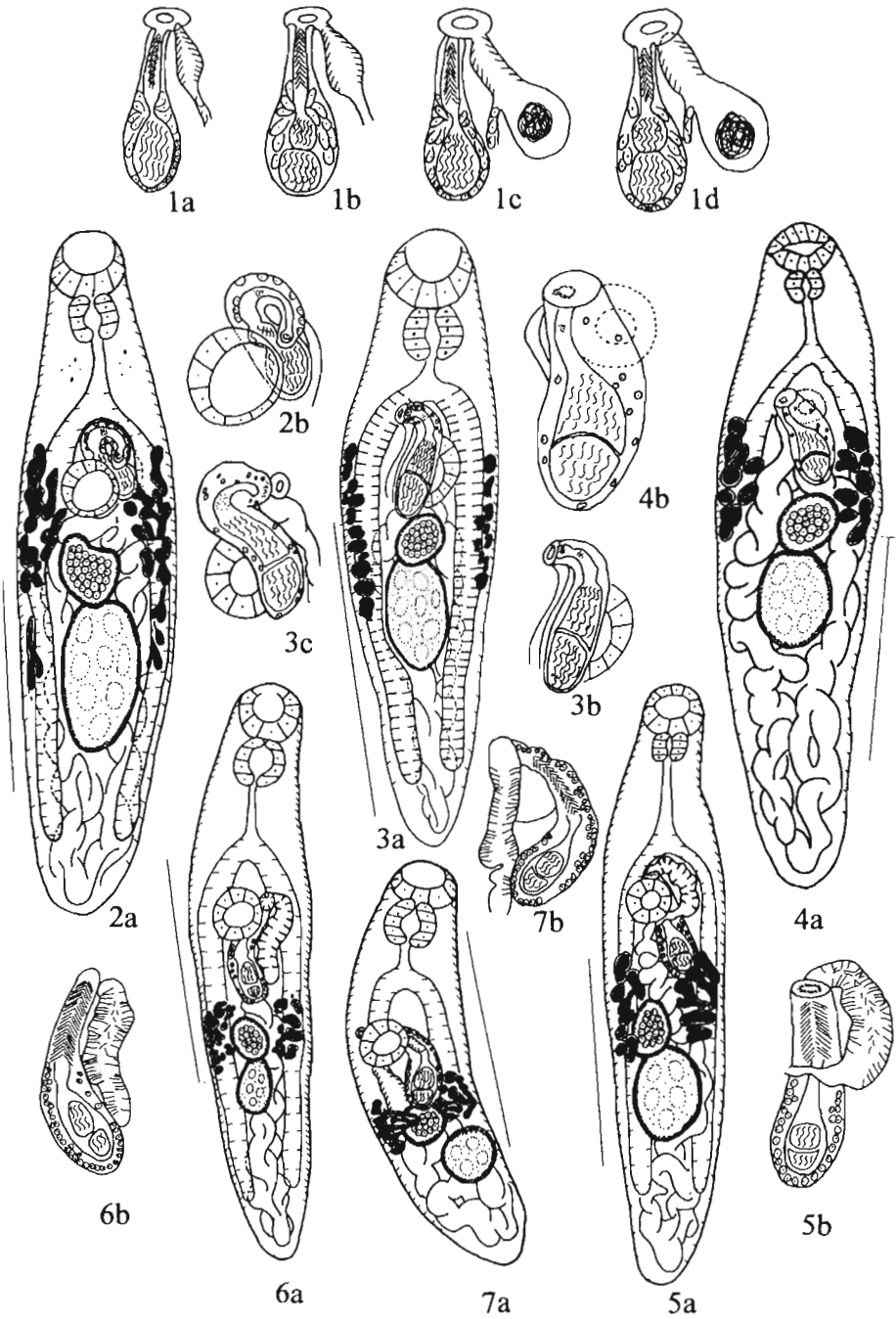
The genus *Hurleytrema* was named by Srivastava (1939) for *H. ovocaudatum* from the intestine of *Teuthis (Siganus) margariifera* (family Siganidae) from Karachi, Arabian Sea. His generic diagnosis emphasized a single testis, caeca extending to near posterior end of the body, a well-developed cirrus sac with an internal oval seminal vesicle, a pars prostatica with prostate glands and a spiny cirrus, vitelline follicles extending laterally from the level of the acetabulum to near posterior end of the body, a lobed pre-testicular ovary, absence of a seminal receptacle, uterus extending to near the posterior end of the caeca and terminating anteriorly in a well-developed spiny metraterm, eggs with a single filament at the anoperculum end, and a genital atrium sinistral to the anterior level of the ventral sucker.

Manter (1942) described *Hurleytrema chaetodoni* from the Chaetodontidae *Chaetodon ocellatus* Bloch and *C. capistratus* Linnaeus and *H. eucinostomi* from the Gerridae *Eucinostomus lefroyi* (Goode) from Tortugas, Florida. Manter stated that *H. chaetodoni* is markedly different from *H. ovocaudatum* in sucker ratio, distribution of vitellaria, longer spiny metraterm and larger eggs, each with a filament at least three times longer than the egg itself. Manter's description indicated the presence of an internal bipartite seminal vesicle and vitellaria of about 20 follicles between the ovary and posterior end of the cirrus sac. In comparing *H. eucinostomi* with *H. ovocaudatum*, he noted also the restricted distribution of the vitellaria which consist of two lateral masses extending between the posterior end of the cirrus sac and the level of the testis. The cirrus sac contains a unipartite seminal vesicle and the uterus enters a bipartite terminal organ with a large posterior spherical vesicle, a smaller swelling just anterior to it followed by a spiny metraterm; where the uterus enters the terminal organ was not indicated. Examination of specimens of



*H. eucinostomi* from Jamaica and Curaçao collected by the senior author shows that the uterus joins the terminal organ near its mid region above the posterior vesicle. The anterior swelling reported by Manter is not evident. Yamaguti (1954) considered the differences cited by Manter of generic significance, removed Manter's two species from *Hurleytrema* and placed them in two new genera *Hurleytrema* (*H. chaetodonti*) and *Pseudohurleytrema* (*P. eucinostomi*). Bravo-Hollis (1956) described *Hurleytrema longitestis* from *Citula dorsalis* and *Selene brevoorti* (Carangidae); Thomas (1959) added *H. trachinoti* from *Trachinotus goreensis* (Carangidae), and Velasquez (1961) *H. malabonensis* from *Parachaetodon ocellatus* (Chaetodontidae). Manter and Pritchard (1961) described *Hurleytrema coronatum* from five species of *Chaetodon* (*C. lineolatus*, *C. auriga*, *C. fremblii*, *C. lunata*, *C. miliaris*) (Chaetodontidae) and from *Naso hexacanthus* (Acanthuridae); Nahhas and Cable (1964) described *H. curacaensis* from *Chaetodon capistratus* and *C. ocellatus* (Chaetodontidae), and Nahhas and Powell (1965) added *Pseudohurleytrema shorti* from *Selene vomer* (Carangidae). In their review of the species known to that date, Nahhas and Powell pointed out that the length of the caeca and distribution of the vitelline follicles may have been over-emphasized as generic characteristics while the terminal reproductive organs relatively ignored. They noted that the seminal vesicle may be single (spherical, ovoid, cylindrical), or bipartite. In the female terminal part, the uterus enters a structure, peculiar to monorchiids, that has been variously referred to as terminal organ, metraterm pouch, metraterm sac, or Looss' organ in European literature. The terminal organ may be a simple, thick- or double-walled structure that is a continuation of the uterus proper or a bipartite structure consisting of a posterior vesicle, usually unarmed, and an anterior part usually spiny; the uterus joins the bipartite terminal organ at some point near or anterior to the junction of the vesicle with the spiny anterior part. Based on this review, Nahhas and Powell concluded that seven of the nine species can be placed in the three existing genera: *Hurleytrema* (seminal vesicle unipartite, terminal organ unipartite Fig. 1a) would include *H. ovocaudatum* Srivastava, 1939 and *H. longitestis* Bravo-Hollis, 1956; *Hurleytrema* (seminal vesicle bipartite, terminal organ unipartite, Fig. 1b) would accommodate *H. chaetodonti* Manter, 1942 and *H. curacaensis* Nahhas & Cable, 1964; *Pseudohurleytrema* (seminal vesicle unipartite, terminal organ bipartite, Fig. 1c) would include *P. eucinostomi* (Manter, 1942), *P. malabonensis* (Velasquez, 1961), and *P. shorti* Nahhas & Powell, 1965. *Hurleytrema trachinoti* Thomas (1959) and *Hurleytrema coronatum* Manter & Pritchard, 1961, characterized by a bipartite seminal vesicle and a bipartite terminal organ would not fit the diagnosis of the three genera. Nahhas and Powell (1965) suggested erection of a new genus, *Parahurleytrema*, (bipartite seminal vesicle and bipartite terminal organ Fig. 1d), to accommodate the two species with *H. trachinoti* as type. Travassos *et al.* (1965) described *Pseudohurleytrema otto* from *Selene vomer*; this species was considered a synonym of *P. shorti* Nahhas & Powell, 1965 by Overstreet (1969) both from the same host species. Description of *P. shorti* appeared earlier in the year.

Overstreet (1969) described *Hurleytrema pyriforme* from *Trachinotus falcatus* (Carangidae) and Kamegai (1970) *Hurleytrema japonicus* from *Chaetodontoplus septentrionalis* (Pomacanthidae); Kaikabad and Bilqees (1981) described *Pseudohurleytrema magna* from an unidentified fish; Madhavi (1974) added *Hurleytrema filiformis* from *Chaetodon pictus* (Chaetodontidae) and Amato (1982) *Hurleytrema catarinensis* from *Trachinotus carolinus* (Carangidae). Shen (1990) described *Hurleytrema hainanensis* from *Tylosurus melanotus* (Belonidae) and Lutjanus *erythropterus* (Lutjanidae) from South China Sea, and Wang and Wang (1993) added *Hurleytrema longus* from



*Siganus fuscescens* (Siganidae) from East China Sea. Based on Nahhas and Powell's concepts of the genera, *H. pyriforme* and *H. catarinensis* should be referred to *Pseudohurleytrema*, becoming *P. pyriforme* n. comb. and *P. catarinensis* n. comb. respectively. *H. japonicus* and *H. filiformis* are properly placed in *Hurleytrema*; *H. japonicus*, described from five specimens, has a sub-median post-acetabular genital pore; Yamaguti (1971) suggested placing it in, but not naming, a separate subgenus. Bilqees (1981) lists *Pseudohurleytrema magna* with a brief description, but gives no reference beyond the authors' names and the year; the cirrus sac has an elongated unipartite seminal vesicle (*Pseudohurleytrema*, *Hurleytrema*) but the nature of the female terminal organ is neither described nor clearly figured. *Hurleytrema hainanensis* Shen, 1990 has a twisted, tripartite seminal vesicle, the terminal organ bottle-shaped and the genital pore on the left margin of the ventral sucker; the relationship of the uterus to the terminal organ is not described; his figure, however, shows the uterus reaching, but not entering, the posterior end of the terminal organ; the genital pore seems to be near the posterior level of the ventral sucker. *H. hainanensis* was compared with *H. ovocaudatum* and *H. longitestis* and distinguished by its rhomboid testis, twisted tripartite seminal vesicle and smaller eggs. The bottle-shaped terminal organ, however, suggests a bipartite condition and, therefore, *Parahurleytrema*; more adequate description of the female terminal organs and the location of the genital pore are needed. *Hurleytrema longus* Wang & Wang, 1993 has a bipartite seminal vesicle (*Hurleytrema* or *Parahurleytrema*) and sac-like terminal organ at the distal end of the uterus; whether the terminal organ is unipartite or bipartite and where the uterus enters the terminal organ are not indicated. These three species are not included in a key presented at the end of the discussion section.

## MATERIALS AND METHODS

A total of 236 fishes, representing 50 species in 32 genera, and 20 families were collected. The fish were obtained from several sources including traps, nets, and spear fishing at reefs and lagoons of Laucala Bay, Suva, a few miles from the laboratory of the Marine Studies Programme (formerly Institute of Marine resources), The University of the South Pacific. Among the fish captured alive, *Heniochus acuminatus*, *H. monoceros*, *Chaetodon citrinellus*, *C. melannotus* (Chaetodontidae) and *Zebrosoma scopas* (Acanthuridae) were found to harbor hurleytremae monorchids. The fish were kept alive

Fig. 1. - Male and female terminal reproductive structures (diagrammatic). a: *Hurleytrema*. b: *Hurleytrema*. c: *Pseudohurleytrema*. d: *Parahurleytrema*.

Fig. 2. - *Hurleytrema fijiensis* n. sp. from *Heniochus acuminatus*. a: holotype, ventral view. b: terminal reproductive organs.

Fig. 3. - *Hurleytrema fijiensis* n. sp. from *Chaetodon citrinellus*. a: dorsal view. b: terminal reproductive organs. c: terminal reproductive organs.

Fig. 4. - *Hurleytrema prevoti* n. sp. from *Chaetodon melannotus*. a: holotype, dorsal view. b: terminal reproductive organs.

Fig. 5. - *Hurleytrema bartolii* n. sp. from *Heniochus acuminatus*. a: holotype, ventral view. b: terminal reproductive organs.

Fig. 6. - *Hurleytrema bartolii* n. sp. from *Heniochus monoceros*. a: ventral view. b: terminal reproductive organs.

Fig. 7. - *Hurleytrema zebrasomae* n. sp. from *Zebrosoma scopas*. a: holotype, ventral view. b: terminal reproductive organs.

in tanks until shortly before examination. After removal from the host, the trematodes were washed in 0.7% saline and studied alive before they were fixed in alcohol-formalin-acetic acid (A.F.A.) under slight cover-slip pressure. The worms were then transferred to a dish, left in the fixative overnight, and stored in vials containing A.F.A. After washing with three changes of 50% isopropanol, the specimens were stained with aceto-carmin, destained in 0.5% acid-alcohol, dehydrated in ascending series of isopropanol, cleared in methylsalicylate, rinsed in xylol, and mounted in Kleermount. Measurements are expressed in micrometers ( $\mu\text{m}$ ) given as a range, with means in parentheses on species represented by 3 or more specimens. Sucker ratio was calculated from the mean of the length and the width, and is expressed with the oral sucker taken as 1. Drawings were prepared by micro-projection and details filled in through microscopic observations. Calculations of prevalence and mean intensity follow the recommendations of Margolis *et al.* (1982) and are indicated, with dates of collection, in the taxonomic summary that precedes description of species. Representatives of all new species are deposited in the Parasite Collection of the United States National Museum (USNPC) and, when sufficient specimens are available, at the British Museum of History, (BMNH), and the Harold W. Manter Laboratory (HWML), Nebraska State Museum, Lincoln. The fish were identified by Mr. Johnson Seeto of the Marine Studies Programme. References used included an unpublished manuscript on fishes of the Fiji Islands, Nelson (1984), Myers (1989) and Randall *et al.* (1990).

## SYSTEMATIC ACCOUNT

### *Hurleytrematoides fijiensis* n. sp. (Figs 2a,b; 3a,b,c)

*Hosts.* - *Heniochus acuminatus* (Linnaeus, 1758) (Chaetodontidae) (Type species); *Chaetodon citrinellus* (Linnaeus, 1758) (Chaetodontidae).

*Prevalence.* - 50% (1 of 2); 66.7% (in 2 of 3).

*Mean intensity.* - 2.

*Dates of collection.* - 4 Feb. 1992, 5 Feb. 1992.

*Holotype.* - USNPC 87585.

*Paratypes.* - BMNH 1997.12.2.1; HWML 39570.

*Etymology.* - The species is named after the Republic of Fiji.

*Description* (based on two specimens from *Heniochus acuminatus*). - Body elongate, rounded at both ends, 1553-1632 long by 330-360 wide at level of ovary. Fore-body 524-539 long; hind-body 909-963 long. Tegument spinose; spines embedded in tegument, extending along entire length, but sparse posterior to testis. Eye spot pigments present lateral to esophagus. Oral sucker terminal 140-153 long by 150-155 wide; ventral sucker 120-130 long by 130-133 wide; sucker ratio 1:0.86. Pre-pharynx absent; pharynx 106-120 long by 110 wide; esophagus 110-143; caeca extending to about two thirds distance between testis and posterior end of body. Testis oblong, 310-340 long by 160-170 wide; cirrus sac arcuate, 183-230 long by 58-60 wide, extending from posterior level of ventral sucker to near intestinal bifurcation, then bending back on left side to anterior level of ventral sucker, and containing bipartite seminal vesicle, short pars prostatica, and cirrus with two clusters of minute spines, one along part of its length, the other at its tip; spines too small to measure. Post-testicular space 415-420. Ovary ovoid to slightly irregular, 120-155 long by 90-100 wide, at mid-body, anterior to and contiguous with testis; seminal receptacle absent; uterine coils extending longitudinally to posterior end

of body, joining tubular unipartite terminal organ on left side of cirrus sac near base of ventral sucker; terminal organ with minute spines along part of its anterior half; vesicle absent. Vitelline follicles extending laterally from near anterior or mid-level of cirrus sac to mid-level of testis. Eggs 25-40 long by 11-14 wide, exclusive of unipolar filament; filament 3-5 times length of egg. Genital atrium small, inconspicuous, mostly sinistral to anterior level of ventral sucker; pore not spined, sub-median, sinistral to anterior level of ventral sucker. Excretory vesicle tubular, extending to anterior level of ovary; pore terminal.

Measurements on four specimens from *Chaetodon citrinellus*: Body 850-1225 (1042) long by 240-290 (265) wide. Fore-body 230-430 (330); hind-body 525-670 (598). Oral sucker 118-150 (135) long by 125-165 (145) wide; ventral sucker 95-125 (110) long by 100-123 (113) wide; sucker ratio 1:0.80. Pharynx 90-105 (98) long by 95-118 (103) wide; esophagus 27-62 long (42). Testis 165-220 (195) long by 72-133 (105) wide; post-testicular space 302-350 (326) long; cirrus sac 205-235 (216) long by 50-60 (54) wide. Ovary globular 57-100 (71) long by 73-90 (78) wide; eggs 37-43 (40) long by 12-18 (15) wide; single unipolar filament, 2-5 times length of egg.

*Remarks.* - The specimens from *Chaetodon citrinellus* (Figs 3a,b,c) agree in major characteristics with those of *H. fijiensis* such as shape of testis, curved or arcuate cirrus sac, a poorly spined cirrus, and similar sucker ratio, but differ slightly in vitelline distribution, follicles not extending anterior to the ventral sucker, in having somewhat larger spines at the tip of the cirrus, an esophagus slightly shorter than the pharynx, and caeca with thick walls and conspicuous lining; the eye spot pigments were difficult to discern and spines were not seen in the terminal organ. Additional differences are seen in some measurements, but on the whole we consider these differences as minor and due to development in different host species. Measurements and figures are given separately to reflect the similarities and differences and as a record for future investigators.

*Hurleytrema fijiensis* is distinguished from all other known species by a combination of the following characteristics: vitelline follicles that extend laterally from near the anterior level of the ventral sucker to mid-testicular level, an arcuate cirrus sac that extends to near intestinal bifurcation and then bends posteriorly on the left side to near anterior level of the ventral sucker, a cirrus with two small clusters of minute spines, one near its middle and the other at its tip, a tubular unipartite terminal organ, with minute spines along its anterior half, a large oblong testis whose length is about twice its width, an esophagus as long as or longer than that of the pharynx, a sucker ratio of 1:0.86, and presence of eye spot pigments.

#### *Hurleytrema prevoti* n. sp. (Figs 4a,b)

*Host.* - *Chaetodon melannotus* Bloch & Schneider, 1801 (Chaetodontidae).

*Prevalence.* - 50% (1 in 1 of 2).

*Mean intensity.* - 1.

*Date of collection.* - 1 Feb. 1992.

*Holotype.* - USNPC No. 87582.

*Etymology.* - This species is named in honor of Professor Georges Prevot in recognition of his contributions to parasitology.

*Description* (based on one specimen). - Body elongate, rounded at both ends, 1453 long by 360 in greatest width. Fore-body 360; hind-body 1030. Tegument spinose,

spines embedded in tegument and extending to near posterior end of body, sparse posterior to testis. Eye spot pigments absent. Oral sucker terminal, 93 long by 125 wide; ventral sucker 63 in diameter. Sucker ratio 1:0.58. Pre-pharynx absent; pharynx 70 in diameter; esophagus 95 long; caeca extending about two thirds distance between testis and posterior end of body. Testis ovoid, 220 long by 153 wide; cirrus sac oblong, 193 long by 85 wide, extending from anterior level of ventral sucker two thirds distance to ovary, containing bipartite seminal vesicle, inconspicuous pars prostatica, and cirrus with two clusters of minute spines, one at its mid-section, the other at its tip. Ovary sub-spherical, 130 long by 120 wide, in mid-body, overlapping slightly anterior level of testis; seminal receptacle absent; uterine coils extending to posterior end of body, joining tubular aspi-nose terminal organ near junction of bipartite seminal vesicle. Vitelline follicles extending laterally from posterior end of ventral sucker to ovario-testicular junction. Eggs 37-40 long by 12-15 wide, exclusive of single unipolar filament, 5-7 times length of egg. Genital atrium thin-walled; pore sinistral, near anterior level of ventral sucker. Excretory vesicle tubular extending to posterior level of testis; pore terminal.

*Remarks.* - *Hurleytrematoides prevoti* shares the characteristic of a poorly spined cirrus with *H. fijiensis* but differs in having a smaller sucker ratio, 1:0.58 compared with 1:0.80-0.86, ratio of ovary to testis, absence of eye spot pigments, and vitelline follicles extending anteriorly to posterior level of ventral sucker; the cirrus sac is shorter and wider, is not curved and does not reach intestinal bifurcation; terminal organ spines are not evident.

*Hurleytrematoides bartolii* n. sp. (Figs 5a,b; 6a,b)

*Hosts.* - *Heniochus acuminatus* (Linnaeus, 1758) (Chaetodontidae) (Type host); *H. monoceros* Cuvier, 1831 (Chaetodontidae).

*Prevalence.* - 50% (10 in 1 of 2); 33.3% (1 of 3).

*Mean intensity.* - 10; 2.

*Date of collection.* - 5 Feb. 1992.

*Holotype.* - USNPC 87584.

*Paratypes.* - BMNH 1997.12.2.2; HWML 39571.

*Etymology.* - This species is named after Professor Pierre Bartoli in recognition of his contributions to marine parasitology.

*Description* (based on seven specimens from *Heniochus acuminatus*). Body elongate, rounded at both ends, 880-1390 (1107) long by 200-325 (253) wide. Fore-body 250-450 (363) long; hind-body 460-1130 (701) long. Tegument spinose; spines embedded in tegument, extending to near posterior end of body. Eye spot pigments absent. Oral sucker 75-120 (101) long by 100-142 (120) wide; ventral sucker 75-95 (85) long by 68-105 (91) wide; sucker ratio 1:0.80 (1:0.76-0.82). Pre-pharynx absent; pharynx 63-90 (74) in diameter; esophagus 60-189 (126); caeca extending almost two thirds distance between testis and posterior end of body. Testis ovoid, 148-250 (188) long by 78-160 (129) wide; cirrus sac straight, 220-315 (262) long by 33-80 (69) wide, extending from anterior level of ventral sucker midway to ovary, containing bipartite seminal vesicle, pars prostatica, and heavily spined cirrus, spines thorn-like, 15-20 (18) each, extending along entire length of cirrus. Post-testicular space 120-430 (247). Ovary ovoid to pear-shaped 83-125 (108) long by 75-110 (90) wide, just posterior to mid-body, anterior to and contiguous with testis; seminal receptacle absent; uterine coils extending to posterior

end of body, joining well-developed unipartite terminal organ near posterior end or mid level of cirrus sac; terminal organ 123-195 (149) long by 23-95 (49) wide, spiny, spines thorn-like 13-18 (15) long, similar in shape to those of cirrus; vesicle absent. Vitelline follicles extending laterally from ovario-testicular junction or slightly beyond to anterior level of seminal vesicle or base of cirrus but not reaching ventral sucker. Eggs 30-40 (35) long by 12-20 (16) wide, exclusive of unipolar filament, each filament 3-5 times length of egg. Genital atrium conspicuous, pore thick-walled, median, immediately anterior to ventral sucker. Excretory vesicle sac-like not reaching posterior testis; pore terminal.

Measurements on two specimens from *Heniochus monoceros*. - Body 1228-1285 long by 250-280 wide. Fore-body 440-450 long; hind-body 660-720 long. Oral sucker 117-123 long by 138-140 wide; ventral sucker 113-115 long by 105-110 wide; sucker ratio 1:0.85. Pharynx 100-105 long by 110-112 wide; esophagus 123-128 long. Testis 123-128 long by 65-70 wide; cirrus sac 248-263 long by 62-65 wide; post-testicular space 310-330. Ovary 80-93 long by 67-70 wide; terminal organ 143-225 long by 50 wide. Eggs 32-40 long by 12-15 wide; single unipolar filament, 4-5 times length of eggs shell.

*Remarks.* - The two specimens from *Heniochus monoceros* (Figs 6a,b) are similar to those from *H. acuminatus* in their major characteristics including the presence of a heavily spined cirrus and unipartite terminal organ, but differ in having a slightly larger pharynx, larger testis relative to the ovary and some measurements, differences, we attribute to development in different host species.

*Hurleytrema bartolii*, found in the type host of *H. fijiensis*, is distinguished from this species and from *H. prevoti* chiefly by the presence of a heavily spined cirrus and a spiny terminal organ.

### *Hurleytrema zebrasomae* n. sp. (Figs 7a,b)

*Host.* - *Zebrasoma scopas* (Cuvier, 1829) (Acanthuridae).

*Prevalence.* - 25% (1 in 4).

*Mean intensity.* - 2.

*Date of collection.* - 6 Feb. 1992.

*Holotype.* - USNPC 87583.

*Etymology.* - This species is named for the host.

*Description* (based on two specimens). - Body rounded at both ends 955-1150 long by 250-260 wide. Fore-body 360-400; hind-body 500-550. Tegument spinose; spines embedded in tegument and extending to near posterior end of body. Eye spot pigments absent. Oral sucker 95-128 long by 133-143 wide; ventral sucker globular 95-100 long by 100 wide; sucker ratio 1:0.78. Pre-pharynx absent; pharynx 97-110 long by 113-123 wide; esophagus 70-80 long; caeca extending to but not reaching posterior end of body. Testis globular or oblong, 113-150 long by 90-107 wide; cirrus sac, curved, well developed, 250-300 long by 62-75 wide, containing bipartite seminal vesicle, pars prostatica, and spiny cirrus. spines 12-20 long covering posterior two thirds of cirrus. Ovary globular 80 long by 67-80 wide; seminal receptacle not evident; uterus extending to near posterior end of body, passing between testis and ovary and in one specimen between ovary and cirrus sac, joining unipartite terminal organ near anterior level of ovary; testis and ovary separated by uterine coils in both specimens; terminal organ well developed, 200-225 long by 58 wide, entire length covered with spines 15-22 long, similar in shape

to those of cirrus; vesicle absent. Vitelline follicles extending laterally from near anterior level of bipartite seminal vesicle to posterior level of ovary. Eggs 40-45 long by 17-22 wide, exclusive of unipolar filament; filament 2-3 times length of egg. Genital atrium near anterior level of ventral sucker; genital pore sub-median, on left side of anterior level of ventral sucker. Excretory vesicle tubular, its anterior extent not determined with certainty, probably to mid-level of cirrus sac; pore terminal.

*Remarks.* - *H. zebrasomae* is similar to *H. bartolii* in having spiny cirrus and unipartite terminal organ but differs by uterine coils separating the testis from the ovary, somewhat shorter esophagus, greater curvature of the cirrus sac and shorter post-testicular space.

## DISCUSSION

The Fijian species fall naturally into two groups based on the degree of spination of the male and female terminal reproductive organs. The first group, consisting of *Hurleytrematoides fijiensis* and *H. prevoti*, are species with reduced spination in the cirrus; the spines are minute and found as two clusters, one about mid-cirrus length, the other at its tip; *H. fijiensis* also has minute spines in its terminal organ whereas these were not seen in *H. prevoti*. The second group consisting of *H. bartolii* and *H. zebrasomae* is characterized by the presence of well developed and conspicuous spines in both the cirrus and the terminal organ. The nature of the spines and the degree of spination in the Fijian species of the first group, and others placed in this genus, require some revision in the generic diagnosis of *Hurleytrematoides* to reflect this condition. *Hurleytrematoides* is, therefore, emended to read: subfamily Hurleytrematinae, cirrus sac with a bipartite seminal vesicle; cirrus spines may be extensive and large or small and restricted in distribution; terminal organ unipartite, without a vesicle, with or without spines.

Inherent in the description of any new taxon based on one or two specimens is the problem of determining degree of variation. Although there are some apparent differences among the specimens, we have taken the conservative attitude of considering the differences between specimens taken from *Heniochus acuminatus* and those from *Chaetodon citrinellus* as minor and due to development in different host species, placing all in *Hurleytrematoides fijiensis*. The same position was taken with respect to those from *Heniochus acuminatus* and *H. monoceros* recognizing both as *Hurleytrematoides bartolii*. *Hurleytrematoides zebrasomae* bears a superficial similarity to *H. bartolii*; the general impression, however, is that the two, obtained from hosts in two different families, Acanthuridae and Chaetodontidae respectively, are distinct.

Two of the four genera we recognize in the subfamily have not been universally accepted. Manter and Pritchard (1961) rejected *Pseudohurleytrema*; Overstreet (1969), Kamegai (1970) and Amato (1982) did not recognize either *Pseudohurleytrema* or *Parahurleytrema*; Walset and Kohn (1987) followed Overstreet and Amato in placing *P. shorti* in *Hurleytrema*. Fischthal and Thomas (1969) accepted *Parahurleytrema* in their redescription of *P. trachinoti* but suggested emending the description of the seminal vesicle to read bipartite or winding, a revision we accept. Yamaguti (1971) also accepted the genus. Madhavi (1974) referred to Nahhas and Powell's concept of the 4 genera but did not indicate acceptance or rejection. Mamaev (1968) placed *Parahurleytrema* in subfamily Hurleytrematinae.



The presence of a unipartite, bipartite or winding seminal vesicle is easy to observe in freshly killed specimens; a bipartite terminal organ is also readily recognizable especially when a vesicle is clearly visible. However, in specimens retrieved from unrefrigerated fish that had been dead for several hours, degenerative morphologic changes often occur. A unipartite terminal organ is the equivalent of a thickened and often spiny metraterm. There would, perhaps, be less confusion if 'metraterm' is used when the structure is unipartite, restricting 'terminal organ' to the bipartite condition. We have, however, followed the traditional terminology. We have accepted the concept of four genera as suggested by Nahhas and Powell (1965) and emended by Fischthal and Thomas (1969). *Hurleytrema coronatum* Manter & Pritchard, 1961 was described to have a well developed terminal organ with a spherical and unarmed vesicle; the point at which the uterus joins the terminal organ was not clearly indicated. We have examined 42 slides of this species (HWML 1603, 1604, 1605, 1606, 1607) from its five hosts; the stain in many of the specimens had faded but several specimens show clearly the presence of a posterior vesicle and the uterus entering the terminal organ just anterior to the vesicle. We have also re-examined a paratype of *Hurleytrema malabonensis* Velasquez, 1961 (USNPC 39475); the stain had also faded and we could not see the details of the terminal organ; this specimen, however, had previously been examined by Nahhas and Powell (1965) when it was still well-stained; based on that observation, the authors at the time transferred it to the genus *Pseudohurleytrema*. In the description of *Hurleytrema japonicus*, Kamegai (1970) also indicated the presence of a posterior vesicle in the terminal organ of *P. malabonensis*. The senior author has been studying monorchiids for several years and is convinced that the female terminal organ in all well-described species clearly falls into two categories: bipartite with a posterior vesicle, and unipartite (metraterm) without a vesicle; absence, presence and size of spines are species specific. There is also some variation in the point of entry of the uterus into the bipartite terminal organ; in some it is immediately anterior to the vesicle, in others it is further up; this characteristic is also species specific. We believe that recognizing two types of terminal organs is not only of generic significance but may also solve several taxonomic problems in monorchiids as a whole. We cite one example relating to the genera *Lasiotocus* and *Proctotrema*. In their redescriptions of *Lasiotocus mulli* and *Proctotrema bacilliovatum*, Bartoli and Prevot (1966) described the former to have a bipartite terminal organ with a posterior vesicle and the latter having a unipartite one, no vesicle, and the uterus entering it at its posterior end. We concur with Mamaev (1968) that *Proctotrema* can be distinguished from *Lasiotocus* by the presence of a unipartite terminal organ compared with a bipartite one. Other characteristics assigned to the two genera have not been satisfactory; many of their species show all degrees of variations and intergradations and have been moved back and forth between the two as well as among others (*Genolopa*, *Paraproctotrema* and *Proctotrema*); these species might be more properly assigned if Bartoli and Prevot's description of the terminal organs of *Lasiotocus* and *Proctotrema* is accepted.

### Key to species

Yamaguti (1971) placed the four hurleytrematine genera in three separate subfamilies. We recognize them all in subfamily Hurleytrematinae as characterized by Manter and Pritchard (1961) and Mamaev (1968). A key to these genera and their species follows:

- 1a. Seminal vesicle unipartite ..... 2
- 1b. Seminal vesicle bipartite or winding ..... 3
- 2a(1a). Terminal organ unipartite; vesicle absent; uterus enters terminal organ at its base ..... *Hurleytrema* 4
- 2b(1a). Terminal organ bipartite; vesicle present; uterus entering terminal organ at some point anterior to vesicle ..... *Pseudohurleytrema* 5
- 3a(1b). Terminal organ unipartite; vesicle absent; uterus entering terminal organ at its base ..... *Hurleytrematoides* 9
- 3b(1b). Terminal organ bipartite; vesicle present; uterus entering terminal organ at some point anterior to vesicle ..... *Parahurleytrema* 17
- 4a(2a). Oral sucker larger than ventral sucker; testis length about 1.5 x width; vitelline follicles extending from posterior level of ventral sucker to near posterior end of body; ovary irregular to trilobed, in posterior half of body; egg filament about same length as egg shell length; cirrus spiny; terminal organ with minute spines ..... *H. ovocaudatum*
- 4b(2a). Oral sucker smaller than ventral sucker; testis length about 5 x width; vitelline follicles extending from mid-ovarian to mid-testicular level; egg filament approximately 1.5 x egg shell length; cirrus spiny; terminal organ tubular with short spines ..... *H. longitestis*
- 5a(2b). Caeca extending to posterior end of body; egg filament 7-8 x egg shell length; cirrus aspinose; terminal organ with spiny aperture ..... *P. malabonensis*
- 5b(2b). Caeca not extending posterior to testis; egg filament 1-4 x egg shell length 6
- 6a(5b). Eye spot pigments absent; egg filament 2-3.5 x egg shell length; cirrus spiny; posterior part of terminal organ aspinose, anterior part spiny ..... *P. shorti*
- 6b(5b). Eye spot pigments present ..... 7
- 7a(6b). Ventral sucker post-equatorial; cirrus spiny; posterior part of terminal organ aspinose, anterior part spiny; ovary at acetabular level; egg filament 2 x egg shell length ..... *P. catarinensis*
- 7b(6b). Ventral sucker equatorial or pre-equatorial ..... 8
- 8a(7b). Body pyriform; caeca extending almost to or slightly past testis; egg filament 2-4 x egg shell length; cirrus spiny; posterior part of terminal organ aspinose, anterior part spiny; vitelline follicles in two lateral clusters between or overlapping ventral sucker and testis ..... *P. pyriforme*
- 8b(7b). Body elongate; caeca reaching slightly beyond mid-body but not past testis; egg filament 1-1.5 x egg shell length; cirrus with few spines at tip; posterior part of terminal organ aspinose, anterior part spiny; vitelline follicles far posterior to ventral sucker, extending laterally from posterior level of terminal organ to ovary or posterior end of caeca ..... *P. eucinostomi*

- 9a(3a).** Body elongate, length about 12 x width; ventral sucker in anterior body fifth; vitelline follicles 6-9 on each side, extending from anterior level of ovary to, but not reaching, base of cirrus sac; testis elongate; cirrus spines bristle-like; terminal organ aspinose ..... *H. filiformis*
- 9b(3a).** Body length less than 7 times width ..... **10**
- 10a(9b).** Egg filament 11-15 x egg shell length; genital pore sub-median, post-acetabular; vitellaria in ovario-acetabular zone; cirrus spiny, spines needle-like, 10 in length; terminal organ tubular, spiny, spines needle-like similar to those of cirrus ..... *H. japonicum*
- 10b(9b).** Egg filament 1-7 x egg shell length; genital pore pre-acetabular or paracetabular ..... **11**
- 11a(10b).** Cirrus spines minute; spines of terminal organ minute; may be absent ..... **12**
- 11b(10b).** Cirrus spines large; spines of terminal organ large ..... **14**
- 12a(11a).** Vitelline follicles extending laterally between posterior level of ventral sucker and ovario-testicular junction; sucker ratio 1:0.58; cirrus sac does not reach intestinal bifurcation; ventral sucker in anterior third of body, smaller than pharynx ..... *H. prevoti*
- 12b(11a).** Vitelline follicles lateral extending anteriorly to at least anterior level of ventral sucker; sucker ratio 1:0.80-0.86 ..... **13**
- 13a(12b).** Eye spot pigments present ..... **14**
- 13b(12b).** Eye spot pigments absent ..... **15**
- 14a(13a).** Testis length about 2 x its width; vitelline follicles lateral, extending from near anterior level of ventral sucker to mid-testicular level; egg filament 2-5 x length of egg shell ..... *H. fijiensis*
- 14b(13a).** Testis length less than 2 x width; vitelline follicles lateral well posterior to ventral sucker, mostly pre-testicular; egg filament 1-1.5 x egg shell length ..... *H. curacaensis*
- 15a(13b).** Vitellaria extending from ovarian level to posterior level of cirrus sac confluent dorsally; filament length 3 x egg shell length; ovary immediately posterior to mid-body, may or may not be separated from testis by uterine coils; sucker ratio about 1:1 ..... *H. chaetodoni*
- 15b(13b).** Vitellaria not confluent dorsally in ovario-cirrus sac zone; oral sucker larger than ventral sucker ..... **16**
- 16a(15b).** Pharynx smaller than ventral sucker; esophagus about same length as or longer than pharynx; cirrus sac straight or slightly curved; ovary contiguous with testis; post-testicular space about one fourth body length ..... *H. bartolii*
- 16b(15b).** Pharynx larger than ventral sucker; esophagus shorter than pharynx; cirrus sac curved; ovary separated from testis by uterine coils ..... *H. zebrasomae*
- 17a(3b).** Body elongate; eggs 32-45 x 16-26, filament 10-15 x egg shell length; ventral sucker pre-equatorial; cirrus spiny; posterior part of terminal organ aspinose; anterior part spiny ..... *P. coronatum*
- 17b(3b).** Body oval-pyriform; eggs 25 x 10, filament 2 x egg shell length; ventral sucker equatorial or post-equatorial; cirrus spiny; posterior part of terminal organ aspinose, anterior part spiny ..... *P. trachinoti*

**Acknowledgements.** - The authors thank the faculty and staff of the Marine Studies Programme at the University of the South Pacific, Suva, Fiji, particularly Professor G. Robin South, Director, for providing the facilities to the senior author to conduct the study, and to Mr. Johnson Seeto for the identification of fish and for his assistance in many other ways. We also thank Dr. J. Ralph Lichtenfels, Biosystematic Parasitology Laboratory, United States Department of Agriculture, Beltsville, Maryland and Mr. M. Sterner, H.W. Manter Laboratory, University of Nebraska, Lincoln for the loan of specimens. Last but not least, the authors wish to thank Professors Rodney A. Bray of the Museum of Natural History, London and Thomas H. Cribb, Department of Zoology, University of Queensland, Australia for their review and suggestions.

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## THE CAMALLANIDAE (NEMATODA) OF INDO-PACIFIC FISHES: TAXONOMY, ECOLOGY AND HOST-PARASITE COEVOLUTION

by

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**ABSTRACT.** - Camallanid nematodes are a common group of gastro-intestinal parasites of marine and freshwater vertebrates worldwide, especially fishes. Fishes become infected when they eat infected microcrustaceans, thus completing the two host life cycle. Though their life cycles may also exhibit a high degree of flexibility, in both number of hosts in the life cycle and host species used, some degree of ecological specificity has been observed. Camallanids are known to cause some pathology, which may drive sexual selection against infected hosts. Thus, despite the potential for high rates of gene flow in marine systems, host parasite coevolution is possible. Methods for the control of camallanids in the aquarium trade and emerging fish farming industry remain largely unexplored. Lastly, their current taxonomy does not appear to reflect evolutionary affinities.

**RÉSUMÉ.** - Les Camallanidae (Nematoda) des poissons indo-pacifiques: taxinomie, écologie et co-évolution hôte-parasite.

Les nématodes de la famille des Camallanidae constituent un groupe de vers parasites gastro-intestinaux des vertébrés marins et d'eau douce du monde entier, notamment des poissons. Ces derniers s'infectent en mangeant des micro-crustacés parasités, les parasites bouclant ainsi leur cycle dans ces deux hôtes. Cependant, leur cycle peut présenter certaines variations, tant par le nombre d'individus-hôtes infectés que par le nombre d'espèces-hôtes parasitées; ces variations affectent aussi le degré de spécificité observé. Les Camallanidae sont connus pour être pathogènes, et ils peuvent être sélectifs sexuellement vis-à-vis de leurs hôtes. Aussi, malgré le fort potentiel de flux géniques dans les écosystèmes marins, la co-évolution hôte-parasite est possible. Les méthodes pour contrôler le développement des Camallanidae en aquariophilie et dans les fermes aquacoles sont largement méconnues. Enfin, la taxinomie actuelle des Camallanidae n'apparaît pas refléter leurs affinités évolutives.

**Key-words.** - Indo-pacific fishes, Nematoda, Camallanidae, ISEW, ISW, Taxonomy, Life cycles, Specificity, Biogeography, Ecology, Pathology, Host-parasite coevolution.

The Camallanidae are parasitic nematodes, most easily recognized by their sclerotized (or, hard) buccal capsules (or, mouth parts) and their red body coloring. These worms are gastro-intestinal parasites of vertebrates, where the worm attaches to the lining of the gastro-intestinal tract (mucosa) using their buccal capsules and feeds on the host's blood. Though they feed on the host's blood, their red coloring comes from their own hemoglobins. Feeding camallanids may cause pathology, potentially leading to both sub-lethal effects and mortality.

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This group of nematodes has not yet been reported from terrestrial systems, and their invasion of these systems may be limited by their dependence on aquatic intermediate hosts. However, they are widespread in both freshwater and marine systems. Camallanids are thought to have originated in freshwater systems, where they are diverse (7 genera) and have a wide host range (amphibians, reptiles, and fishes), and then have spread to marine systems, where diversity is low (4 genera) and their host range is more restricted (fishes). Though species diversity in marine systems appears to be lower than in freshwater systems, this group may still be one of the more common parasites in marine fishes. For example, in French Polynesia, 5 species of camallanids were found to infect 15% of coral reef fishes examined (representing 66% of nematode infections) from a survey of nearly 300 fishes representing 30 families (S. Morand, unpubl. data).

From above, it would appear that camallanids are well studied. However, apart from their primary taxonomy, our knowledge of this group remains limited, especially for the marine genera. Here, I will review the current state of knowledge on marine camallanids of fishes, including the following topics: Life Cycles and Specificity, Zoogeography, Community Ecology, Pathology, Host-Parasite Coevolution, Methods for their Control, and Taxonomy and Terminology.

### Life cycles and specificity

Only two life cycles of marine camallanids have been completed. One unidentified *Spirocamallanus* was found to have a two host life cycle: the fish *Acanthurus triostegus* (Acanthuridae) is the definitive host and an unidentified amphipod is the second host. Herbivorous fishes (such as *A. triostegus*) may become infected by accidentally ingesting infected amphipods associated with algae. The second known life cycle is that of *S. platycephali*, which uses a fish definitive host (whiting, or *Sillago* spp.) and a microcrustacean intermediate host (both copepods and amphipods may be used). Freshwater camallanids also have 2 host life cycles. In these worms, the definitive vertebrate host becomes infected when it eats an infected copepod, though paratenic hosts are also common. However, their life cycles may also show incredible flexibility, with trophic transfer between serial paratenic hosts and vertebrate hosts being possible for the same worm.

Specificity is known to be low among marine species for their definitive hosts. For example, *Spirocamallanus monotaxis* has been found in 12 fish species of 8 families from 4 orders and *S. istiblenni* has been reported from 8 fish species of 6 families from 2 orders. The freshwater parasite, *Camallanus lacustris*, has even been reported from several different vertebrate classes (e.g., fishes, birds, and snakes) and the freshwater *C. oxycephalus* has been reported from 52 species of fish. Though there is no information available on the specificity of marine camallanids for their intermediate hosts, specificity appears to be low for the intermediate hosts of freshwater species, so long as it is a copepod. Trophic transfer, however, may have some influence on the low host specificity of these worms. For example, the freshwater parasite *C. lacustris*, mentioned above, found in snakes and birds is probably acquired by eating infected fishes.

Some degree of ecological specificity, however, is evident. As implied above, freshwater and marine habitats appear to have largely distinct camallanid faunas. In marine systems Rigby and Adamson (1997) have noted further ecological specificity; *S. istiblenni* and *S. monotaxis* were found in carnivorous fishes while *S. chaimha* and *S. colei* were found in herbivorous fishes of French Polynesian coral reefs. Further, while *S. istiblenni* and *S. monotaxis* were not restricted to any reef type, the worms in herbivorous fishes were restricted; e.g., *S. chaimha* was only found in fishes on protected reefs and



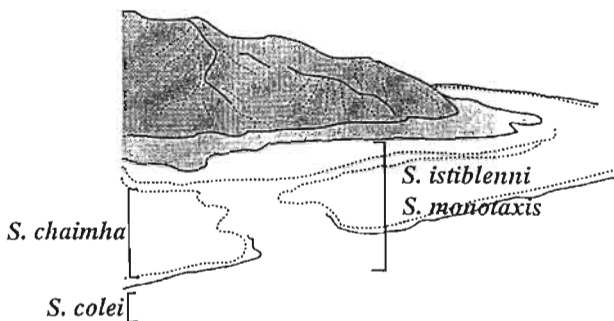


Fig. 1. - Distribution of different *Spirocamallanus* spp. on the reefs of Moorea, French Polynesia. Species found in herbivorous fishes are on the left and species found in carnivorous fishes are on the right.

*S. colei* was only found in fishes on exposed reefs (Fig. 1). Such ecological specificity may give insight into species specific physiological and life cycle requirements, which have yet to be investigated.

### Biogeography

As the non-pelagic habitats in the South Pacific consist of a series of widely distributed small islands and the presumptive sources of colonizing species are the larger land masses to the west (Australia and Asia), dispersing to the consecutively remoter islands to the east should become progressively more difficult. Thus, in the islands of the South Pacific, the number of non-pelagic species decreases from west to east. This has been observed in most non-pelagic organisms (e.g., fishes, corals, echinoderms, plants, etc.), but not in camallanids. Some camallanids have extensive ranges (e.g., *S. istiblenni*, *S. monotaxis*, and *Camallanus carangis* have all been reported from widely disparate locations). The low host specificity and potential for trophic transfer of worms in this family may have permitted them to attain their extensive ranges; i.e., this may allow them to infect pelagic intermediate, paratenic, and definitive hosts, potentially increasing their dispersal ability and reducing the potential for a cline in species numbers. However, this is only based on numbers of species reported in the literature and may reflect sampling effort bias.

### (Infra)community ecology

Sadly, this appears to be poorly investigated with respect to any camallanid. Rigby *et al.* (1997) attempted to do so in French Polynesia, but only one other parasite occurred in the intestines (*Scolex polymorphus*), with few double infections, and so no effects on distribution within the host were observed. The site of infection in the camallanids of marine fishes is generally the intestines, where there are often other nematodes and digeneans; thus, some interactions should be possible. Unfortunately, interactions with other helminths appear to have been overlooked in general. However, we can compare the prevalence of camallanids in surveys of fishes from French Polynesia (39 fish infected of 260 sampled, or 15%) (S. Morand, unpubl. data) to that of Australia (9 fish infected of 185 sampled, or 5%). Since overall parasite abundance is much greater in Australia, though there may be other factors involved, the lower prevalence of camallanids (Fisher's exact test, 2 tailed  $P = 0.0006$ ) may suggest that camallanids do not compete well against other gastro-intestinal parasites within a host; i.e., camallanids are better colonizers.

### Pathology

Worms of this family attach to the mucosa of the gastro-intestinal tract where they feed on the host's blood. In high intensity infections of freshwater camallanids, it has been suggested that these worms may cause intestinal ulceration, excessive production of goblet cells and mucous in the intestines, anemia, emaciation, listlessness, and death. I have also received an unpublished autopsy of 3 angelfishes infected with *Camallanus cotti* from Purdue University. Infections consisted of 25-75 worms per fish and caused ulceration and hyperaemia of the anus, emaciation, and anemia in their hosts (S. Bowman, pers. comm.). Though it appears that further study is necessary, there can be no doubt that these worms are pathogenic to their hosts to some degree.

Pathology in intermediate hosts has also not been investigated in marine camallanids. Among the freshwater systems examined, infection by camallanid larvae in copepods may cause higher mortality and reduced motility.

### Host-parasite coevolution

In marine systems, many organisms have pelagic larvae, which may lead to increased dispersal. This would appear to be true for both camallanids (they may use pelagic microcrustaceans as intermediate hosts) and their definitive fish hosts. Thus, the offspring of both the host and the parasite may not encounter the same parasite and host species, respectively, and may respond to differing selective pressures. However, given the above pathology of the worms, host-parasite coevolution may occur where there is less dispersal of both host and parasite genotypes. This would seem more likely to occur in freshwater systems, or in some closed or dense marine systems; e.g., closed lagoons or fish farms. Indeed, it has been shown that male guppies (*Poecilia reticulata*) parasitized by *Camallanus cotti* display less often to females and that females may prefer unparasitized males. Such parasite effects on sexual selection support the hypothesis that animals should choose mates on the basis of genetic resistance to parasites, which may produce frequency dependent co-adaptation cycles with corresponding genotypic variation. Thus, camallanids may cause sexual selection for resistance traits in their hosts, leading to fitness differences among host genotypes and possible evolutionary responses in the host population. Guppies are already well investigated for life-history evolution in vertebrates and have been shown to respond to environmental variables and predation.

### Control

This is an area that has, as yet, received very little attention but will probably become more important as fish farming increases. Indeed, in areas where there are large numbers of fish confined to a small space, parasite intensities may reach very high levels and cause pathology in their hosts. This applies equally to marine fish farms, from which this has already been reported. However, control is still a problem in that nothing has been specifically tested against camallanids in the scientific literature. In the popular press, levamisole and piperazine citrate are recommended though there are other untested antinematodals available (e.g., fenbendazole and mebendazole). Controlling the intermediate hosts would also seem logical, which should only be possible in aquarium reared fishes, though methods for controlling aquatic invertebrates may also be toxic to their fish hosts.



Fig. 2. - A: *En face* view of the buccal capsule of a camallanine, showing the division between the two valves. B: *En face* view of the buccal capsule of a procamallanine, showing that the capsule is not divided.

### Taxonomy and terminology

Higher taxonomy in the family Camallanidae is based on gross differences in buccal capsule morphology. Two subfamilies are currently recognized, based on the construction of the buccal capsule: worms with an undivided buccal capsule are placed in the Procamlaninae while worms with a divided buccal capsule are placed in the Camallaninae (Fig. 2). Genera in the Procamlaninae have traditionally been distinguished on the basis of finer differences in buccal capsule morphology (e.g., the presence or absence of various "ornamentations" on the inner surface of the capsule), with several genera recognized in each subfamily. Recently, however, it has become apparent that the traditional genera may be both questionable and paraphyletic, and various authors have noticed inconsistencies in the classification of the camallanid genera. Preliminary morphological phylogenies appear to reveal that the Camallaninae is indeed a monophyletic clade derived from the Procamlaninae. The Procamlaninae, however, does not appear to be monophyletic. Also, some of the existing genera in each of the above subfamilies do not appear to be monophyletic (M. Rigby and S. Koulianos, unpubl. data). However, attempts of molecular phylogenies of this group will be hampered by the presence of a DNA co-precipitate that interferes with PCR, as is common in other parasitic nematodes. A phylogeny may also be used to address the origin of this group; i.e., is it a diverse freshwater group with limited success in marine environments or is it a marine group that underwent much greater radiation in freshwater systems?

Species level differences are also often unclear. The lack, or often inaccessibility, of many type specimens hampers their investigation. Additionally, confusion over terminology of the male caudal features may have created some artificial species. Therefore, to avoid further confusion, I define and illustrate the following caudal features: pre-anal papillae (pedunculate papillae attached to the alae anterior to the anus), adanal papillae (sessile papillae not attached to alae with 1 pair anterior and 1 pair posterior to anus), postanal papillae (pedunculate papillae attached to alae posterior to anus), and phasmid (pedunculate sensory structure attached to the alae between posteriormost papillae and posterior extremity, often confused with papillae) (Fig. 3). To help differentiate between species, mapping the positions of these structures may prove useful. Rigby and Adamson (1997) developed the following formula for the positions of the caudal structures:

$$\left( \frac{\text{Distance of structure from posterior}}{\text{Distance of anteriormost union of alae from posterior extremity}} \right) \times 100$$

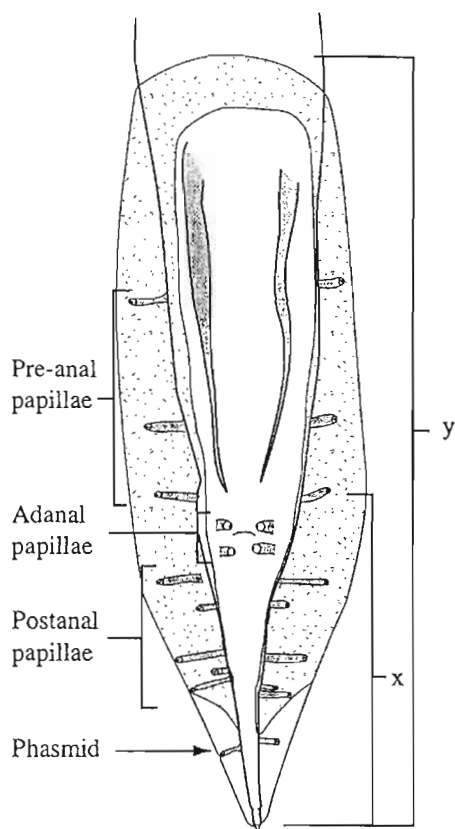


Fig. 3. - Ventral view of the male caudus, showing the terminology used for the papillae and how to calculate the relative position of a papilla.

For example, for the 3rd pre-anal papilla in figure 3, this would be:

$$\left(\frac{x}{y}\right) \times 100$$

This should allow statistical comparisons between species (instead of qualitative), hopefully facilitating species determinations. The methods given in Appendix 1 also facilitate observation of many of the features of taxonomic importance. My co-workers and I have used these methods to describe the camallanids of French Polynesia and other islands in the Pacific. From this, I have developed a key to the camallanid genera found in Pacific fishes and to the species found in the coral reef fishes of French Polynesia (Appendix 2).

## CONCLUSIONS

The marine Camallanidae would appear to be rather sparsely investigated with many gaps in our knowledge left to be filled. At present, camallanids are of little economic importance (except in the aquarium fish trade and tropical fish farming, where they may cause mortality) and remain primarily of academic interest. However, their simple and

flexible life cycles should make them easy to maintain in the lab (e.g., *Camallanus cotti* has spread over a large portion of the world and aquarium infections have proven very difficult to treat), where they may be valuable in studies of host-parasite interactions and evolution among vertebrates.

**Acknowledgements.** - The work leading to this paper was supported by NSERC A1464 to Dr. J. C. Holmes and, in part, by CNRS and EGPN while in French Polynesia, and SNSF 3100-046759.96/1 to Dr. J. Jokela. I am grateful to Steve Bowman, Drs. W. F. Font, T. H. Cribb, T. L. Deardorff, J. Jokela, and S. Koulianos for their assistance.

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### Appendix 1. - Methods for examination of nematodes.

Methods for the examination of nematodes have recently been reviewed by Moravec (1994). Here, I expand upon his review.

**Fixation.** - The most commonly used methods for fixation of camallanids are 1) hot 70% EtOH and 2) hot formalin. However, neither technique adequately straightens or clears specimens. Thus, clearing with lactophenol, acetic acid, or creosote may be required. Fixation with Berland's fluid (1 part glacial acetic acid: 9 parts pure formalin) until straight (about 10 s) yields consistently straight, clear, and highly transparent specimens. Specimens should then be washed at least once in 70% EtOH before being stored. Moravec (1994), however, cautions that acetic acid may damage weakly sclerotized structures.

It should be noted that none of the above methods are suitable for future genetic analysis of specimens. Specimens will need to be preserved separately for genetic analysis.

**Storage.** - Specimens are best stored in 70% EtOH with at least 5% glycerin (to prevent damage to specimens if the alcohol evaporates) and 0.05% phenol (to prevent decay).

**Examination.** - Stored specimens may be examined as temporary whole mounts in glycerin following evaporation in EtOH and glycerin. Clearing is not necessary if Berland's fluid was used for fixation. Staining may even obscure some structures. *En face* views of the anterior end are best performed by placing a drop of warm glycerine jelly on the underside of a coverslip and positioning the detached anterior end as desired. *En face* examinations yield the best results when done using Nomarsky phase microscopy.

- |                |   |                                   |
|----------------|---|-----------------------------------|
| <b>1a.</b>     | Buccal capsule divided into 2 valves, lined with longitudinal ridges (Fig. 2A)<br>..... Subfamily Camallaninae  | <b>2</b>                          |
| <b>1b.</b>     | Buccal capsule undivided, lined or unlined with spiral ridges (Fig. 2B)<br>..... Subfamily Procamallaninae  | <b>3</b>                          |
| <b>2a(1a).</b> | Primarily found in (epi)pelagic fishes, tridents long, tridents united well below posterior margin of buccal capsule, prongs of tridents much shorter than total trident length (Fig. 4A) .....             | <i>Oncophora</i> sp.              |
| <b>2b(1a).</b> | Primarily found in coral reef fishes, tridents short, tridents united near level of posterior margin of buccal capsule, prongs of tridents approximately equal to half total trident length (Fig. 4B) ..... | <i>Camallanus carangis</i>        |
| <b>3a(1b).</b> | Buccal capsule smooth on inner surface (Fig. 4C) .....  | <i>Procamallanus</i> sp.          |
| <b>3b(1b).</b> | Buccal capsule lined with spiral ridges on inner surface (Fig. 4D)<br>.....   | <i>Spirocamallanus</i> spp.       |
| <b>4a(3b).</b> | Primarily found in herbivorous fishes, female tail blunt or tapered .....   | <b>5</b>                          |
| <b>4b(3b).</b> | Primarily found in carnivorous fishes, female tail ends in finger like digit (Fig. 5 A) .....   | <b>6</b>                          |
| <b>5a(4a).</b> | Buccal capsule with more than 20 ridges, cuticle surrounding vulva raised and rugose, female tail rounded (Fig. 5 B) .....  | <i>Spirocamallanus chaimha</i>    |
| <b>5b(4a).</b> | Buccal capsule with fewer than 20 ridges, cuticle surrounding vulva not raised, female tail moderately constricted terminally but finger like digit absent (Fig. 5 C) .....                                 | <i>Spirocamallanus colei</i>      |
| <b>6a(4b).</b> | Second pre-anal papilla 35-48% of distance from 1st to 3rd pre-anal papilla (or, closer to 1st pre-anal papilla) .....  | <i>Spirocamallanus monotaxis</i>  |
| <b>6a(4b).</b> | Second pre-anal papilla 60-75% of distance from 1st to 3rd pre-anal papilla (or, closer to 3rd pre-anal papilla) .....  | <i>Spirocamallanus istiblenni</i> |



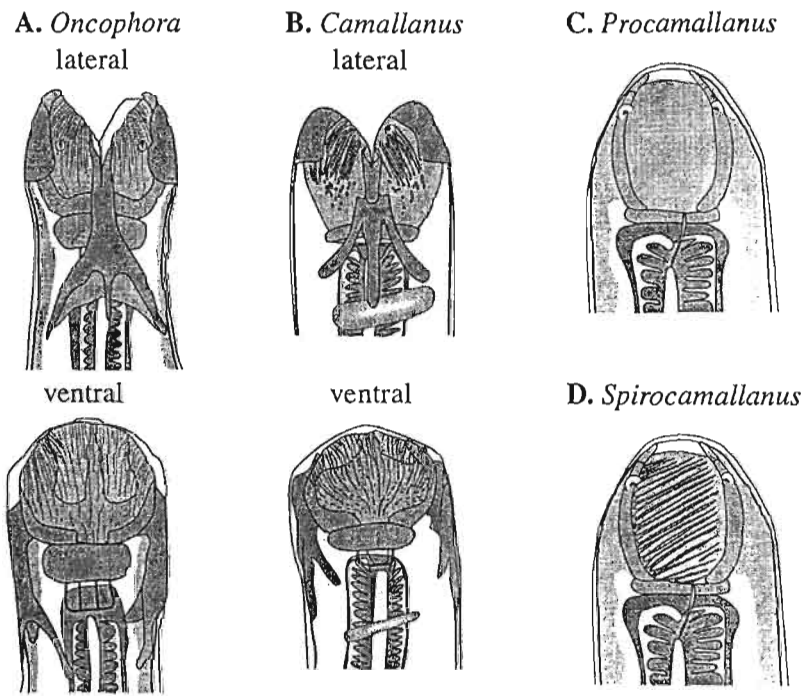


Fig. 4. - A: *Oncophora melanocephala*, ventral and lateral views of the male buccal region. B: *Camallanus carangis*, ventral and lateral views of the female buccal region. C: *Procamallanus*, female buccal region. D: *Spirocamallanus istiblenni*, female buccal region.

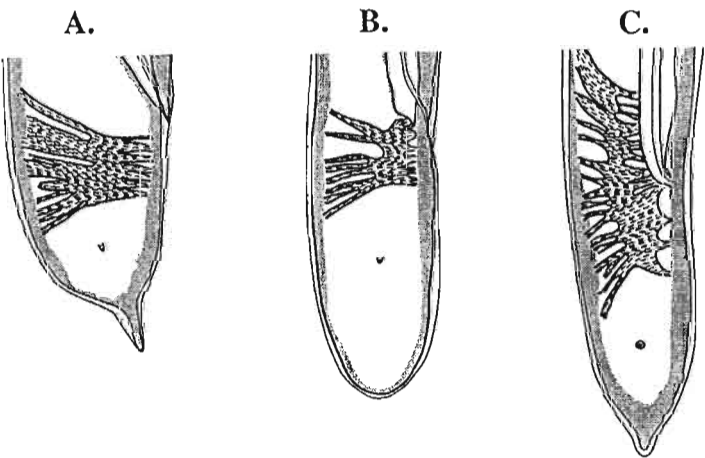


Fig. 5. - A: Lateral view of female tail of *Spirocamallanus istiblenni*, showing a terminal finger like digit. B: Lateral view of female tail of *S. chaimha*, showing a rounded female tail. C: Lateral view of female tail of *S. colei*, showing a female tail that is moderately constricted terminally but without a finger like digit.

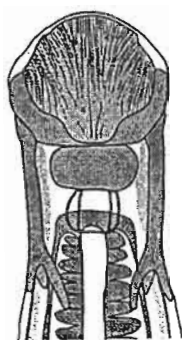


Fig. 6. - Ventral view of the male buccal capsule of *Camallanus aotea* (after Slankis and Korotaeva, 1974).

It should be noted that only species currently recognized within the genus *Oncophora* is *Oncophora melanocephala*. Though this genus is only found in the Atlantic and Mediterranean, there is probably a member of this genus in the Pacific. *Camallanus aotea* was described from the South Pacific, however, it would appear to be more properly placed in the genus *Oncophora* (Fig. 6).

## Workshop 2

### DATABASES ON INDO-PACIFIC FISHES

Chairperson: **Rainer FROESE**

- ALBERT J.S., FROESE R., BAUCHOT R. & H. ITO. - Diversity of brain size in fishes: Preliminary analysis of a database including 1174 species in 45 orders. 647-656

*Diversité de la taille de l'encéphale chez les poissons: analyse préliminaire d'une base de données comprenant 1174 espèces appartenant à 45 ordres.*

- LABROSSE P., LETOURNEUR Y., KULBICKI M. & F. MAGRON. - FISHEYE: 657-672  
A new database on the biology and ecology of lagoon and reef fishes of the South Pacific. Example of its use on the ecology of commercial herbivorous fishes.

*FISHEYE: une nouvelle base de données sur la biologie et l'écologie des poissons récifaux et lagunaires du Pacifique Sud. Exemple de son utilisation en écologie des poissons herbivores commerciaux.*

- SHAO K.T., CHEN J.-P. & S.-C. WANG. - Biogeography and database of marine fishes in Taiwan waters. 673-680

*Biogéographie et base de données des poissons marins de Taïwan.*



## **DIVERSITY OF BRAIN SIZE IN FISHES: PRELIMINARY ANALYSIS OF A DATABASE INCLUDING 1174 SPECIES IN 45 ORDERS**

by

James S. ALBERT (1), Rainer FROESE (2), Roland BAUCHOT (3) & Hironobu ITO (1, 4)

**ABSTRACT.** - Absolute and relative values of brain weight are now available for 1174 species of fishes, representing 45 taxonomic orders. The original FishBase "Brains" data was assembled by the research team of Bauchot and colleagues, to which the present report adds data for species representing several additional major taxonomic groups. This database is part of the FishBase 97 package which provides researchers with a tool to explore the functional meaning of absolute and relative brain size diversity, in comparison with phylogenetic position, life history mode, locomotion, habitat, and other behavioral parameters. Several results are provided as an example of the use of these data. Galeomorph sharks and batoid rays possess the largest brains among fishes, and elongate forms with anguilliform locomotion (e.g., hagfishes, lampreys, true eels, carapids, zoarcids) possess the smallest relative brain sizes. Among teleost fishes, Osteoglossomorphs possess the largest relative brain sizes. Brain size correlations with oxygen consumption suggest that larger brains consume proportionately more oxygen, or that active fish with higher metabolic rates have larger brains.

**RÉSUMÉ.** - Diversité de la taille de l'encéphale chez les poissons: analyse préliminaire d'une base de données comprenant 1174 espèces appartenant à 45 ordres.

Les poids absolus et relatifs de l'encéphale sont connus chez 1174 espèces de poissons appartenant à 45 ordres. Ce rapport complète les données de départ rassemblées par l'équipe du Pr. R. Bauchot pour la partie "Encéphales" de FishBase. La base de données présentée est intégrée à la version FishBase 97 qui fournit aux chercheurs un outil pour explorer le rôle fonctionnel de la diversité des tailles absolue et relative de l'encéphale. Cette exploration peut se faire par rapport à la position phylogénétique, au mode de vie, à la locomotion, à l'habitat et à d'autres paramètres comportementaux. Plusieurs exemples d'exploitation des données sont présentés. Les plus gros encéphales sont observés chez les requins Galéomorphes et les raies. Les formes allongées à locomotion anguilliforme (myxines, lamproies, anguilles, carapidés, zoarcidés) possèdent, en taille relative, les encéphales les plus petits. Chez les Téléostéens, ce sont les Ostéoglossomorphes qui possèdent l'encéphale le plus gros. L'étude des corrélations entre la consommation d'oxygène et la taille de l'encéphale suggère deux hypothèses. La première est qu'une consommation supérieure d'oxygène est nécessaire lorsque l'encéphale est plus gros. La deuxième est que les poissons possédant une activité et un taux métabolique plus élevé ont un encéphale plus grand.

**Key-words.** - Myxiniiformes, Petromyzontiformes, Chondrichthyes, Actinopterygii, Dipnoi, Coelacanthi-formes, Brain size, Comparative morphology, Physiology, Ecology, Evolution.

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There have been numerous studies documenting patterns of brain size diversity within circumscribed groups of fishes (Table I). These investigations have been enriched and also complicated by the enormous diversity of fishes, which constitute the majority of vertebrate species. In one form or another fishes inhabit most of the aquatic habitats on earth, surviving and reproducing in the intense pressure and darkness of the deepest marine trenches (10 km), subzero Antarctic waters, torrential mountain rivers, desert hot-springs and nearly anoxic swamps. In addition to changes in body size and shape, the brains of fishes have also been modified during the course of phylogeny to suit these various life history modes and environments. It is therefore sometimes possible to use brain size difference among fishes to investigate the relationships of brain evolution with physiological and other morphological data, and to draw inferences on their ecology and life-history requirements (Bauchot *et al.*, 1989).

The "Brains" table of the FishBase 97 (Froese and Pauly, 1997) database allows inferences of this sort. This database allows researchers to address the following kinds of questions: What kinds of variation in brain size are present within and among closely related species? How much of the range of phenotypic variation observed among all fishes is represented in individual groups? What ecological and life history variables are associated with changes in relative brain size? This report provides a brief introduction to the "Brains" table of the FishBase 97 package, and illustrates several examples of how it may be used as a research tool.

Table I. - Sources of data used from surveys of brain size diversity within circumscribed groups of fishes.

Taxon	Source
Actinopterygii	Platel <i>et al.</i> , 1977; Northcutt and Braford, 1980
Catostomidae	Miller and Evans, 1965
Chaetodontidae	Bauchot <i>et al.</i> , 1989a
Cichlidae	Huber <i>et al.</i> , 1997; van Staaden <i>et al.</i> , 1997
Craniata	Crile and Quiring, 1940
Cyprinidae	Evans, 1952; Masai <i>et al.</i> , 1982; Kortschal and Junger, 1988; Kortschal and Palzenberger, 1992
Elasmobranchii	Bauchot <i>et al.</i> , 1976; Northcutt, 1977
Escoidae	Means and Lannoo, 1996
Gymnotiformes	Albert, 1999
Myxinoidei, Petromyzontidae	Ebinger <i>et al.</i> , 1983; Platel and Delfini, 1981, 1986
Notothenioidea	Eastman and Lannoo, 1996
Osteoglossidae	Bauchot <i>et al.</i> , 1994
Sarcopterygii	Northcutt <i>et al.</i> , 1978
Teleostei	Ito, 1978; Bauchot and Bauchot 1986; Bauchot <i>et al.</i> , 1979, 1989b; Ridet and Bauchot 1990a, b, 1991

## MATERIALS AND METHODS

The majority of the data on brain size was assembled by the research team of Bauchot and colleagues (Bauchot *et al.*, 1979, 1989, 1994; Ridet and Bauchot, 1990a, 1990b), to which the authors have added data for species representing several additional taxonomic groups. Data are presently available for 1174 species of fishes, representing 45 taxonomic orders, including 14 perciform suborders (taxonomy following Eschmeyer *et al.*, 1997). Most of these data are available in the "Brains" table of FishBase 97 (Froese and Pauly, 1997), and some of the newer additions will be available in FishBase 98, due for release in 1998. The fishes examined were collected from all over the world over a period of many years, from such localities as the Hawaiian and Marshall Islands, New Caledonia, Queensland (Australia), Northern Marianas islands (Guam and Rota), the Philippines, Sulawesi (Indonesia), southwest India, Mauritius and Réunion, Gulf of Oman, northern Red Sea, Senegal, the Caribbean, the Sea of Cortez (Mexico), the Amazon river of Brazil and Peru, the Orinoco river of Venezuela, Honshu (Japan), many localities in the United States, France, and the North Atlantic sea. Data newly reported in the present study are listed in table II.

All fish were weighed before removal of the brain and their standard and/or total lengths taken. The brain was cut from the spinal cord at the first spinal nerves, the meninges removed, the brain blotted and weighed, and then preserved in Bouin solution. Brain size data were obtained by immersing fixed brains in fixative and weighing on an analytical balance. The accuracy of ten repeated measurements estimated on a small brain (c. 0.2 mg) was  $\pm 0.5\%$ . Cranial nerves were transected at the base of the brain and neither they nor the meninges, blood vessels or choroid plexus were included in the weights. The original research reported for this report was performed under guidelines established by the Japanese Society for Neuroscience.

Some specimens for some species were acquired from museum collections, commercial aquarium dealers and fishers. These include the megamouth shark *Megachasma plagios* (Ito *et al.*, 1999), the lungfish *Protopterus annectens*, the bichir *Polypterus palmus*, the sturgeon *Acipenser transmontanus*, several osteoglossomorphs, cypriniforms, tetras (Characiformes), catfishes (Siluriformes), American electric fishes (Gymnotiformes), ricefishes and needle fishes (Beloniformes), and zoarcoid percomorphs. Additional data were compiled from the literature for hagfishes, lampreys, some sharks, and several groups of non-teleost ray-finned fishes (Ito *et al.*, unpubl. data). The phylogeny of fishes used in analyses of higher level taxa was compiled from numerous sources (see Stiassny *et al.*, 1997, and references therein).

Juveniles have a larger brain relative to body weight than adults (Bauchot *et al.*, 1979) and therefore adult fishes were preferred for weight determinations. Data were also available for a size series in some species, ranging from juveniles to large adults, thus allowing the study of ontogenetic changes in brain size. In the Brains table records are presented with the following fields: Genus and Species, Species Code, Stock Code, Locality, Number, Year, Body Weight (g), Brain Weight (mg), Encephalization Coefficient 1 ( $EC_1$ ), Encephalization Coefficient 2 ( $EC_2$ ), Standard Length (SL in cm), Total Length (TL in cm), and Remarks. Encephalization coefficient 1 is a calculated field;  $EC_1 = \text{Log}_{10}[\text{brain weight} \times 1000 / \text{body weight}]$ . Encephalization Coefficient 2 is also a calculated field, which standardizes brain weights over the range of body sizes according geometric considerations:  $EC_2 = \text{Log}_{10}[\text{brain weight} \times 1000 / \text{body weight}^{2/3}]$  (Snell, 1892; Bauchot *et al.*, 1989; Froese and Pauly, 1997).

Table II. - Body and brain weight data in grams for 41 species representing 14 orders newly reported in the present study. Taxonomy following Eschmeyer (1994). Maximum values reported for species with multiple records. N = number specimens examined.

Genus / species	Order	Body	Brain	N
<i>Megachasma plagios</i>	Lamniformes	1,004,000.000	19.100	1
<i>Protopterus annectens</i>	Lepidosireniformes	7.660	0.224	1
<i>Polypterus palmus</i>	Polypteriformes	65.140	0.096	1
<i>Acipenser transmontanus</i>	Acipenseriformes	3,520.000	0.650	6
<i>Lepisosteus osseus</i>	Lepisosteiformes	35.860	0.200	1
<i>Heterotis niloticus</i>	Osteoglossiformes	343.300	1.600	2
<i>Osteoglossum bicirrhosum</i>	Osteoglossiformes	2.900	0.100	1
<i>Scleropages formosus</i>	Osteoglossiformes	11.000	0.300	1
<i>Scleropages jardinii</i>	Osteoglossiformes	17.300	0.300	3
<i>Pollimyrus isidori</i>	Mormyriiformes	2.070	0.115	1
<i>Esox lucius</i>	Esociformes	11,500.000	0.285	1
<i>Umbra krameri</i>	Esociformes	6.440	0.028	1
<i>Oncorhynchus mykiss</i>	Salmoniformes	280.000	0.428	1
<i>Parodon gesteri</i>	Characiformes	3.070	0.043	1
<i>Pygocentrus nattereri</i>	Characiformes	8.480	0.058	1
<i>Adontosternarchus balaenops</i>	Gymnotiformes	7.020	0.117	2
<i>Apteronotus albifrons</i>	Gymnotiformes	34.800	0.171	5
<i>Apteronotus leptorhynchus</i>	Gymnotiformes	25.210	0.150	10
<i>Brachyhyopomus brevirostris</i>	Gymnotiformes	11.460	0.090	1
<i>Gymnotus carapo</i>	Gymnotiformes	96.000	0.266	5
<i>Orthosternarchus tamandua</i>	Gymnotiformes	13.040	0.219	1
<i>Porotergus compsus</i>	Gymnotiformes	14.150	0.127	14
<i>Steatogenys duidae</i>	Gymnotiformes	5.650	0.078	1
<i>Steatogenys elegans</i>	Gymnotiformes	11.960	0.093	3
<i>Sternarchella orthos</i>	Gymnotiformes	12.150	0.131	5
<i>Sternarchella sina</i>	Gymnotiformes	32.800	0.258	5
<i>Sternarchorhamphus nuelleri</i>	Gymnotiformes	22.100	0.208	1
<i>Sternarchorhynchus curvirostris</i>	Gymnotiformes	13.000	0.179	1
<i>Diplomystes nahualbutensis</i>	Siluriformes	2.010	0.047	3
<i>Ictalurus punctatus</i>	Siluriformes	54.000	0.162	10
<i>Noturus flavus</i>	Siluriformes	23.200	0.088	10
<i>Pimelodus pictus</i>	Siluriformes	2.350	0.057	10
<i>Dermogenys megarhamphus</i>	Beloniformes	0.611	0.019	1
<i>Nomorhamphus towoeti</i>	Beloniformes	0.589	0.018	1
<i>Oryzias celebensis</i>	Beloniformes	0.452	0.015	3
<i>Oryzias dancena</i>	Beloniformes	0.242	0.014	3
<i>Oryzias latipes</i>	Beloniformes	0.315	0.012	5
<i>Oryzias natanensis</i>	Beloniformes	0.614	0.016	5
<i>Oryzias marmoratus</i>	Beloniformes	0.644	0.017	5
<i>Oryzias profundicola</i>	Beloniformes	0.594	0.016	5
<i>Lycenchelys</i> sp.	Perciformes	4.520	0.009	2



The analysis of interspecific quantitative data is the subject of a large literature which this report does not attempt to review (see Ridet and Bauchot, 1990b; Harvey and Pagel, 1991; Purvis and Rambaut, 1995). Here it is sufficient to note that methods which treat species values as statistically independent points violate the assumption of sampling independence, because closely related species tend to share characters through common descent rather than only through independent evolution. This phylogenetic correlation may result in characteristics co-occurring among species without there being any interesting reason. Among fishes, for instance, there is a strong tendency for species with spines to be covered with scales, a spurious correlation resulting from the non-independence of species.

## RESULTS AND DISCUSSION

Relative brain weights among the major groups of fishes are expressed as minimum convex polygons in figure 1. Figure 2 shows all data points in FishBase 97, including 3218 records representing 1174 species, plotting  $EC_1$  against the  $\log_{10}$  of body weight. Figure 2 illustrates an output option of Fishbase 97, which can superimpose the relative brain weight of individual taxa (in this case gymnotiform electric fishes as black dots) on the cloud of all FishBase 97 data points (gray dots).

The qualitative results of our analysis of brain size differences among the major groups of fishes is similar to that of van Dongen (1998). Data on brain size diversity in FishBase 97 range over 5 orders of magnitude, from that of the gobiid teleost *Pandaka lidwilli* (body weight = 0.1 g, brain weight = 1.6 mg) to that of the lamniform shark *Megachasma pelagios* (body weight = 1,004 kg, brain weight = 19.1 g). Since body size diversity in fishes ranges by about 7 orders of magnitude brain size diversity is less than that of body size diversity. A study of the data in FishBase 97 also reveals that much of the diversity in brain size in fishes occurs among relatively closely related species (i.e., confamilials). There are, however, also trends that emerge in comparisons at higher taxonomic levels. Gnathostomes in general possess much larger brains than do the jawless fishes. Among fishes, chondrichthyans possess the largest brains, both absolutely and in proportion to their body sizes (Fig. 1). Especially enlarged brains have evolved in at least two groups of cartilaginous fishes; galeomorph sharks and batoid rays (Northcutt, 1977).

Figure 3 depicts comparisons of relative brain weight in 9 teleost taxa for which a range of brain size data are available. In this plot relative brain weight is measured by  $EC_2$ . To assess the effect of body size differences on relative brain size, data for Osteoglossidae are combined from two species (*Heterotis niloticus* and *Scleropages jardinii*) and for *Acanthurus* from 12 species. These data demonstrate that brain size in many teleosts scales with body size very close to the theoretical  $2/3$  expected from geometrical considerations (Snell, 1892). As a result, the size of the brain in adult specimens of many large bodied teleosts (e.g., *Thunnus albacares* and *Gadus morhua*) is attained by following a common growth curve.

Figure 3 also illustrates that certain teleost species with elongate bodies possess relatively smaller brains at larger body sizes than do most teleosts, a pattern resulting from the disproportionate growth of their bodies with respect to their heads. This is especially pronounced in fishes which use anguilliform locomotion (Webb and Weihs, 1983), including true eels (Anguilliformes; e.g., *Conger conger*) and zoarcoid percomorphs (e.g.,

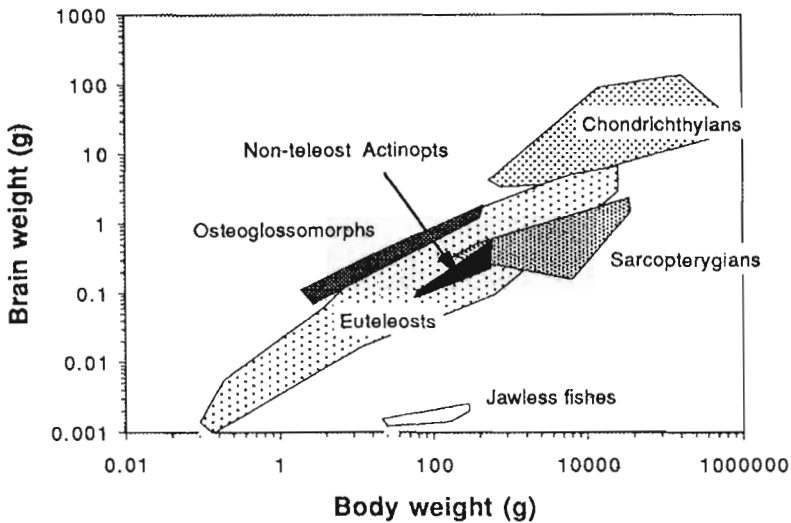


Fig. 1. - Relative brain weight among the major groups of fishes, expressed as minimum convex polygons. Data sources are summarized in tables I and II.

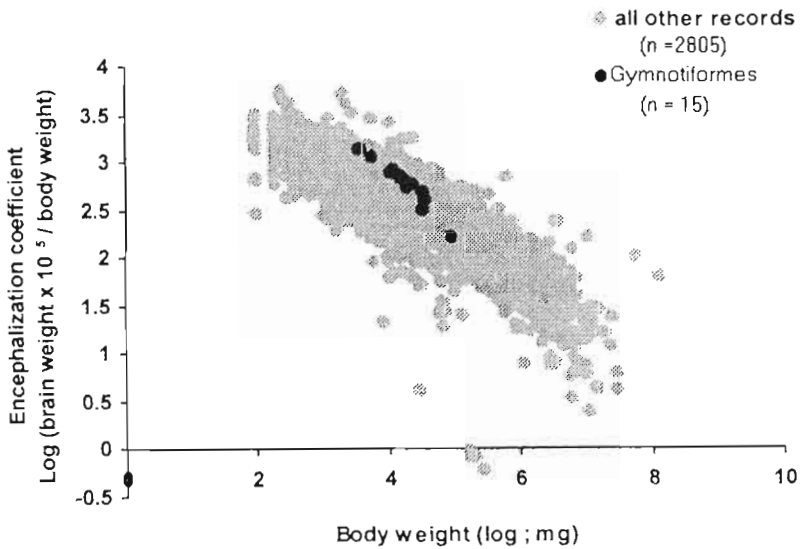


Fig. 2. - Relative brain weight of gymnotiform electric fishes (black dots) superimposed on the cloud of all FishBase 97 data points (gray dots). Use of double log format following Bauchot *et al.* (1989b) and Froese and Pauly (1997). A total of 3218 records are plotted, representing 1174 species. Relative brain weight measured by Encephalization Coefficient 1 ( $EC_1 = \text{Log}_{10}[\text{brain weight} \times 1000 / \text{body weight}]$ ).

*Anarhichas lupus*, *A. denticulatus*). Elongate fish species which do not use anguilliform locomotion (e.g., the gymnotiform electric fish *Porotergerus compsus*) do not possess

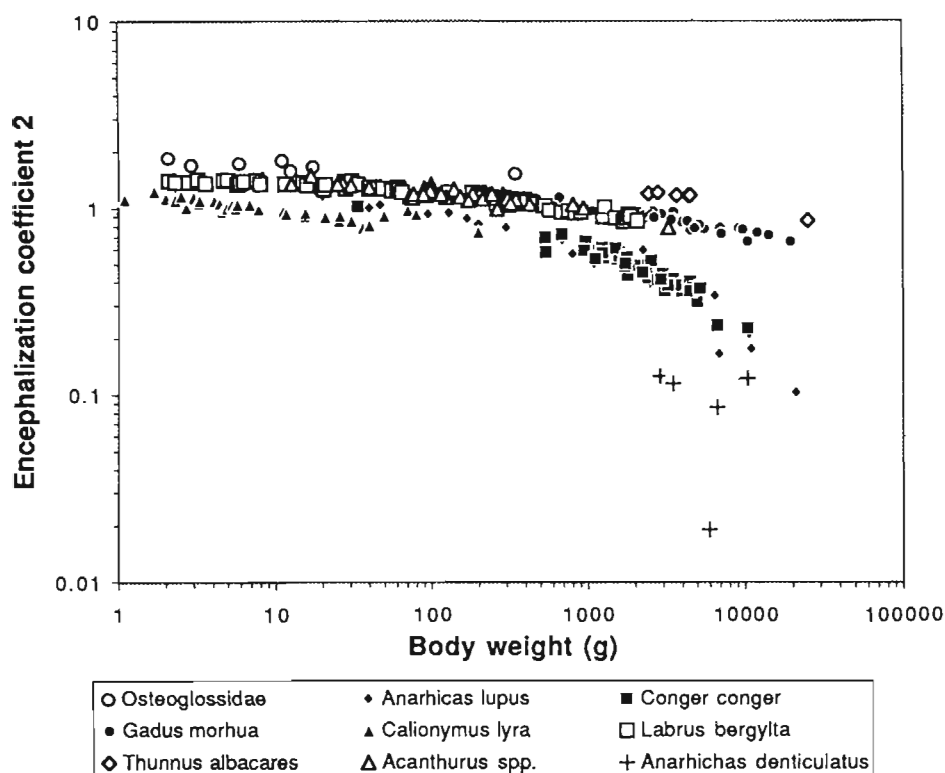


Fig. 3. - Comparisons of relative brain weight in some teleost species for which a range of brain size data are available. Relative brain weight measured by Encephalization Coefficient 2 ( $EC_2 = \text{Log}_{10}[\text{brain weight} \times 1000 / \text{body weight}^{2/3}]$ ).

relatively smaller brains. This pattern resembles the “fat old fish” phenomenon, which refers to a tendency in fishes close to their maximum size to divert from isometric growth and increase fatty tissues rather than muscles, bones, and brain. The data shown for *Thunnus albacares* and *Gadus morhua* do not show this effect due to the absence of very large specimens.

Figure 4 depicts another application of the FishBase 97 package, which permits the comparison of data from different tables. In this case, data from the “Brains” are related with metabolism, using data from the “Oxygen” table. Both datasets present measurements on individual fish which are strongly correlated with body weight. The slope (exponent) of the log-log relationship between oxygen consumption and relative brain weight over all available data was used to correct the individual values for the effect of weight. In this plot each data point represents the average of the available values for species with at least three records. Figure 4 shows that there is sizable variance in the regression of oxygen consumption against brain weight. These data do not refute the hypotheses that large brains consume proportionately more oxygen, or that active fish with higher metabolic rates have larger brains. The variance in these data is anticipated to be reduced once the Oxygen table has been thoroughly proofed.

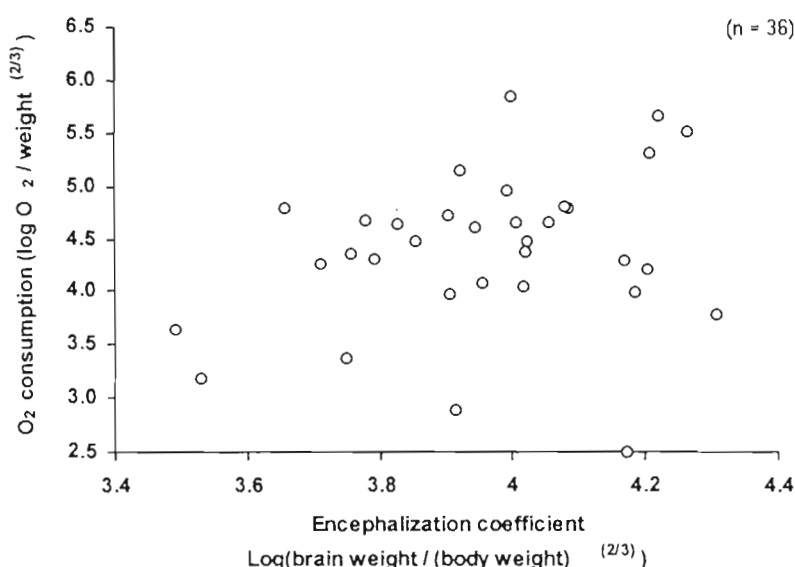


Fig. 4. - Brain weight vs oxygen consumption for several teleost species. Brain weight measured by Encephalization Coefficient 2 (Fig. 3). Note that these data do not refute the hypotheses that large brains consume proportionately more oxygen, or that active fishes with higher metabolic rates have larger brains.

**Acknowledgements.** - We thank M.-L. Bauchot, M. Diagne, J.E. Randall, and J.-M. Ridet for their valuable assistance in assembling the original Brains database, and J.-C. Hureau for transferring the file format. We also thank K. Matsuura and G. Shinohara (National Science Museum, Tokyo), and D. Nelson (Museum of Zoology, University of Michigan) for the loan of specimens, and K. Fukushima for technical assistance. D. Pauly, N. Sawai, N. Yamamoto, M. Yoshimoto and T. Yuri provided useful comments on the manuscript. FishBase is funded by grants from the European Commission. This paper is ICLARM Contribution No. 1426.

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## FISHEYE: A NEW DATABASE ON THE BIOLOGY AND ECOLOGY OF LAGOON AND REEF FISHES OF THE SOUTH PACIFIC. EXAMPLE OF ITS USE ON THE ECOLOGY OF COMMERCIAL HERBIVOROUS FISHES

by

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**ABSTRACT.** - FISHEYE is a database on lagoon and reef fishes of the South Pacific. This data base yields information on the biology of species (reproduction, diet, length-weight relationships, etc.) and ecology of fish communities (species richness, density, biomass, trophic structure, etc.). To date only half of the available data is in the data base. These data are mainly from New Caledonia. In a near future data from French Polynesia, Tonga, Flores will be added and in a more distant future data from Fiji and Samoa should be available. Information is extracted from FISHEYE by requests based on three keys: zone, species and type of analysis (biology or ecology). Some possible uses of FISHEYE are illustrated by the case study of commercially important herbivorous fishes. Species richness, density and biomass of three families, Acanthuridae, Scaridae and Siganidae are compared for three regions (SW lagoon, Ouvéa atoll and North lagoon) of New Caledonia and three subregions of the North lagoon. Three biotopes are considered, barrier, intermediate and fringing reefs. Between and within region differences are found mainly for the density and biomass of these fish. The North lagoon displays the highest biomasses for all three families and the highest density for Acanthuridae. In general, there is a decline from the barrier reef towards the fringing reefs, except for Siganidae which show the opposite trend. A more detailed study was performed on five major species belonging to these families: *Acanthurus blochii*, *Naso unicornis*, *Scarus microrhinos*, *S. ghobban* et *Siganus argenteus*. Finally, the importance of these herbivores within the trophic structure of reef fish were analyzed with FISHEYE. These results confirm the increase of densities and biomasses of reef fishes according to oceanic influence. The between and within regional differences could be related to geographical factors (terrestrial and oceanic influences) and fishing pressure.

**RÉSUMÉ.** - FISHEYE: une nouvelle base de données sur la biologie et l'écologie des poissons récifaux et lagunaires du Pacifique Sud. Exemple de son utilisation en écologie des poissons herbivores commerciaux.

FISHEYE est une base de données sur les poissons lagunaires et récifaux du Pacifique Sud. Elle fournit des informations sur la biologie des espèces (reproduction, alimentation, relations taille-poids, etc.) et l'écologie des communautés (richesse spécifique, densité, biomasse, structure trophique, etc.). Plus de la moitié des données actuellement disponibles sont intégrées dans la base. Elles concernent principalement la Nouvelle-Calédonie. Dans un futur proche, viendront s'ajouter les données de Polynésie Française, de Tonga, des Îles Flores, et dans un futur plus lointain, celles de Fidji et des Samoa américaines. Les informations sont extraites de FISHEYE par des requêtes basées sur trois clés: zone, espèce, type de traitement (biologie ou écologie). Certaines possibilités d'utilisation de FISHEYE sont illustrées par l'étude d'un cas: celui des poissons herbivores commerciaux. La richesse spécifique, la densité et la biomasse de trois familles, les Acanthuridae, les Scaridae et les Siganidae sont comparées dans trois régions (lagon sud-ouest, lagon nord et atoll d'Ouvéa) et sous-régions de la Nouvelle-Calédonie ainsi que sur les trois principaux biotopes récifaux que sont les récifs barrières, intermédiaires

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res et frangeants. Les différences inter- et intra-régionales concernent principalement les densités et les biomasses. Le lagon nord montre les plus fortes valeurs de biomasse pour les trois familles et la densité la plus élevée pour les Acanthuridae. Globalement, les différences entre biotopes montrent, pour les trois paramètres étudiés, un gradient décroissant du récif barrière vers le récif frangeant pour les Acanthuridae et les Scaridae. La tendance inverse est observée pour les Siganidae. Une analyse plus détaillée est présentée pour les espèces principales appartenant à ces trois familles: *Acanthurus blochii*, *Naso unicornis*, *Scarus microrhinos*, *S. ghobban* et *Siganus argenteus*. Enfin, la place des herbivores dans les structures trophiques de l'ensemble des communautés de poissons récifaux a pu aussi être analysée grâce à FISHEYE. Ces résultats confirment ceux qui ont été obtenus dans d'autres régions du Pacifique Sud, notamment ceux qui sont relatifs à l'augmentation des densités et des biomasses en fonction de l'influence océanique. Les variations inter-régionales observées peuvent être reliées à des situations géographiques (influences terrigènes et océaniques) et à des pressions de pêche différentes.

Key-words. - Lagoon fish, Reef fish, ISEW, South Pacific, Database, Biology, Ecology.

From 1995 to 1998, ORSTOM assessed the resources of demersal lagoon and reef fishes of commercial interest, at the request of New Caledonia's Northern Province. This resulted in large data sets on the biology and ecology of these fish (Labrosse *et al.*, 1996, 1997a; Letourneur *et al.*, 1997b). Such knowledge will be of interest to a wide range of audience but especially those concerned with the development and management of the lagoon's biological resources, such as fisheries and environmental agencies, professional fishermen and even anyone looking for information on the subject.

So far, the results of this kind of studies have been always presented in a « traditional » format, namely in the form of technical reports. Although such reports contain valuable information, they are often ignored or read by few people. Other problems are the limited distribution of these reports and the great difficulty in using these data sets for comparisons with other studies in an interactive way.

It was thought that the best way to answer the problems of this type of data set was a computerised client / server application, accessible through the Internet. FISHEYE was therefore developed as a solution, a locally based, user friendly and easily upgradable database where the information could be rapidly and easily available to its end-users.

The similarities between the sampling methods we used for the North Province survey and those used by other ORSTOM studies carried out over the last 14 years (Kulbicki, 1997a) suggested the possibility of integrating into FISHEYE large data sets covering other areas such as the south-west lagoon of New Caledonia (Kulbicki *et al.*, 1987, 1991, 1996), the atoll of Ouvéa (Kulbicki *et al.*, 1994), the Chesterfield Islands (Kulbicki *et al.*, 1990), French Polynesia (Harmelin *et al.*, 1997), Tonga (Matoto *et al.*, 1996), Flores in Indonesia (Kulbicki, 1996), etc. These data cover various methods, the most used being underwater visual census (UVC), trawling, gill netting and rotenone poisoning. Future research by ORSTOM in new areas (e.g., Fiji) will be integrated into this data-base as data becomes available. We are also hoping to awake the interest of other scientists in the Pacific and have their data sets included in FISHEYE as is the case of data from the American Samoa by Green (1996).

In this paper, some of the potential uses of FISHEYE are illustrated by a case study. The distribution patterns of three families of fish, Acanthuridae, Scaridae and Siganidae, which are mainly herbivores, will be investigated. These fish are of major economical and ecological importance in New Caledonia as well as in a number of Indo-Pacific countries and therefore the example illustrate both scientific and management implications. Similar



informations could be drawn for many other species or families (FISHEYE holds information on more than 500 species at present). The aim of the present article is not to explore all the possibilities offered by this data-base, but rather to demonstrate the practical usefulness of the FISHEYE concept. Some of the planned future developments of FISHEYE will also be briefly outlined in the discussion.

## **PRESENTATION OF FISHEYE**

### **Basic facts on FISHEYE**

At the moment FISHEYE has information on a number of fish parameters. The summary presented in table I gives an idea of the present and future potential use of this data base. This indicates that less than half of the information collected is accessible at the moment. Altogether there is data on over a thousand species, but the quality and quantity of the information is variable from one species to another.

### **Access to FISHEYE**

FISHEYE is available in two versions, one in French the other in English. They can be consulted on the following URL (universal resource locator):

English version: [http://noumea.orstom.nc/BASE/FISHEYE/presentation\\_en.html](http://noumea.orstom.nc/BASE/FISHEYE/presentation_en.html)

French version: [http://noumea.orstom.nc/BASE/FISHEYE/presentation\\_html](http://noumea.orstom.nc/BASE/FISHEYE/presentation_html)

### **Structure of FISHEYE**

FISHEYE can be described as a dynamic data base. This means that it performs calculations at the time of the user's request, using the most recently entered data. In order to retrieve information from FISHEYE, the user has to perform a request, each request being based on three major interactive choices or selections (Fig. 1): Geographical area, species and type of computation. Usually, geographical area and species are chosen first. This may involve several areas simultaneously and one or several species or families. In a second step the user chooses the type of information needed among a set of processing schemes which encompasses three fields: biology, ecology and population or community structures. The details of the various computations used are available in an interactive mode in the data base. In many instances, the data processing options include the possibility of analysing the information as a function of given parameters, such as fish size, depth or season.

### **Geographical choice**

At the moment, only data from New Caledonia are in the data base, but we have in store data from Tonga, French Polynesia, American Samoa, Chesterfield Islands and Flores (Indonesia). Within a country, i.e., New Caledonia, the user may choose to select one or several areas (there are 5 areas available at the moment for New Caledonia) and within each area one or several zones, which are further divided into subzones.

### **Species choice**

Species are listed alphabetically and by family. The user may select either species or families, knowing that a selection may include an unlimited number of species or families. At the moment, over 500 species belonging to 86 families are available.

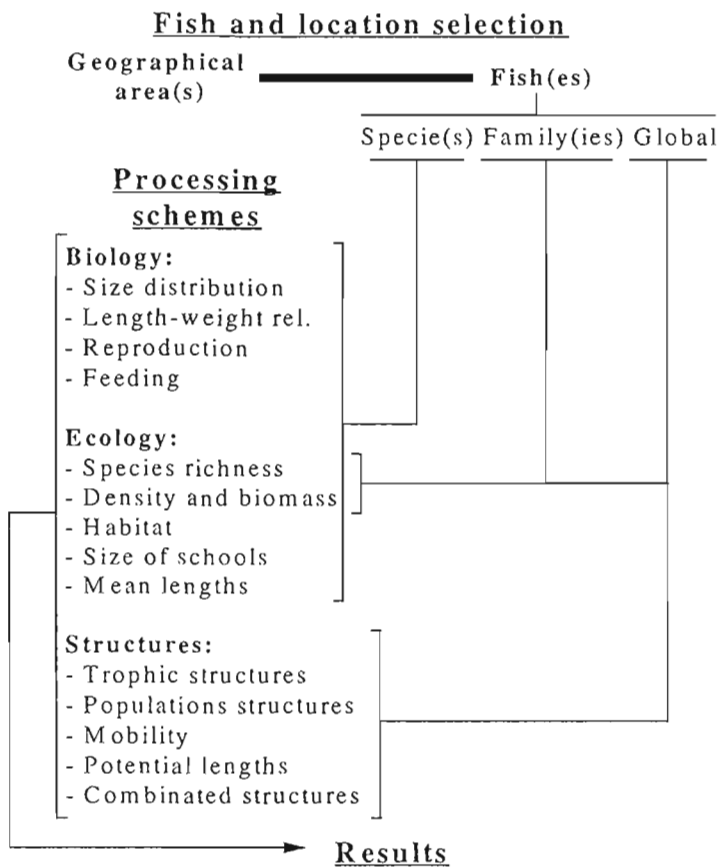


Fig. 1. - Path for a request in FISHEYE. The user chooses a geographical area, then the species and finally the processing scheme.

Table I. - Major types of informations available on FISHEYE (UVC = underwater visual censuses).

	Number of records		Number of species concerned	
	In 1997	In store	In 1997	In store
<b>Sampling</b>				
UVC	170 000	180 000	500	700
Experimental fishing	10 000	15 000	200	900
<b>Biology</b>				
Size	150 000	120 000	500	900
Reproduction	5 000	10 000	120	250
Diet	8 000	25 000	200	370
<b>Ecology</b>				
School size	150 000	120 000	500	900
Habitat	150 000	120 000	500	900
Distribution	180 000	150 000	500	900

### **Computation choices**

Fourteen different modes of data processing are available, divided into 3 categories:

- The biology of species: size distribution, length-weight relationships, reproduction and diet.
- The ecology of species and/or families: species richness, biomass, density, habitat, school size, average specimen size.
- The structures of communities for all species: trophic, demographic, mobility, size potential, and combinations among them.

### **Size distribution**

Data on the size of fish has been obtained from estimates by divers using UVC as well as fish measured from experimental fishing. Based on this data, it is possible to obtain size distribution for any combination of area or biotope for each species.

### **Length-weight relationships**

These are used for estimating the weight as a function of size for the species and area selected. It is based on length-weight relationships that have been calculated existing data in FISHEYE. They are of the form:  $W = a \times L^b$ . Parameters  $a$  and  $b$  are estimated through the least square regression using the following logarithmic transformation:  $\ln(W) = \ln(a) + b \times \ln(L)$ .

### **Reproduction**

The sex and level of sexual development of fish caught are determined by macroscopic examination of the gonads. The data can then be retrieved as a function of size, season and depth of capture for any given species.

### **Diet**

Average composition of food intake for each species is determined by examination of the stomach contents of fish caught during experimental fishing. The results are expressed as percent volume and can be retrieved as a function of size, sex, season or depth for any combination of species and area.

### **Density and biomass**

Density and biomass are obtained from two sources; underwater visual censuses (UVC) and trawling. For UVC these parameters are calculated using the method of line-transect (Buckland *et al.*, 1993). For trawls, densities and biomasses are estimated from the area swept by the trawl, a catchability coefficient of 1 being used.

### **Habitat**

During underwater visual censuses, the characteristics of the substrate and the cover by living organisms (algae, hard and soft coral, etc.) are recorded. From these data it is therefore possible to estimate the average composition of the substrate and its living cover for a given species, for the area chosen.

### **School size**

This information is obtained from UVC data, by dividing the total number of fish observed by the number of occurrences for a given species. This parameter is therefore a crude estimate of school size, and can be obtained per biotope, depth or fish size.

**Average sizes**

The average size of fish is given for UVC data for different depth strata.

**Species richness**

This indicates the number of species within one family or fish assemblage. It is expressed as the mean number of species per station within the area chosen. This parameter is given separately for each biotope and for the different sampling methods (UVC and experimental fishing).

**Trophic structure**

Most species have a varied diet, including several kinds of food: nekton (piscivores), macro-invertebrates (> 20 mm), micro-invertebrates, zooplankton, other planktonic organisms, macro-algae, micro-algae, detritus, corals. The contribution of each species to any of the trophic groups can be evaluated. It is a function of the percentage of this type of food in its total food intake. For example, a 100 g fish whose diet includes 20% nekton contributes 20 g to the biomass of the piscivores. The other 80 g are distributed proportionately to the respective percentages of the other components of its diet. This structure is available only in multispecies selection.

**Potential size**

Fish are classified in relation to the average size of the adult specimen known from available literature. For example, if the average adult size of *Lethrinus atkinsoni* (yellow tailed emperor) is 32 cm, and even though some individuals may measure only 5 cm while some others reach 45 cm, the species is classified as being in the 30-50 cm category. It is available only in multispecies selections.

**Demographic structure**

Each species is classified according to its biological characteristics, such as lifespan, age at sexual maturity, etc., into 6 different categories. Despite the imprecisions due to the limited knowledge for certain species, the proportion of each category within a fish assemblage can be useful for understanding its potential evolution when faced with particular events (fishing activities, pollution, cyclone, etc.).

**Mobility**

The different species of lagoon and reef fishes display various degrees of mobility. A fish assemblage observed while diving may be broken down according to the degree of mobility of the different species encountered (territorial, sedentary, mobile small radius, mobile large radius).

**Combined structures**

This function presents cross-tables from trophic structures, demographic structures and mobility. In a near future, school size and average size will also be integrated in this function.

### CASE STUDY: COMMERCIAL HERBIVOROUS FISHES

In order to illustrate some of the potentials of FISHEYE, we have chosen to analyse the distribution and major ecological traits of three families of commercially important fish, Acanthuridae, Scaridae and Siganidae, which are mainly herbivorous. Three regions the North lagoon, South West lagoon and Uvea atoll and three subregions of the North lagoon were selected. For each region and subregion the species richness, average density and biomass per family and for some major species were obtained. For these selected species size distribution and school size were also retrieved from FISHEYE. Within the Northern Province the spatial distribution of species richness for the major biotopes is examined as well as the contribution of herbivores to the trophic structure.

For the three families considered, species richness (species/station), density (fish/m<sup>2</sup>) and biomass (g/m<sup>2</sup>) are given for the three regions and the three subregions (Table II). Acanthuridae and Scaridae had the same species richness (5.3 to 5.7 species/transect) and density (0.11 fish/m<sup>2</sup>) whilst Siganidae had lower species diversity (1.47 species/transect) and lower densities (0.024). Scaridae had biomasses twice larger than Acanthuridae and 20 times larger than Siganidae. Regional differences were found in terms of density and biomass. However, within region differences were of the same magnitude than regional differences. Ouvea was characterized by low levels of Siganidae, low densities of Scaridae and high densities of Acanthuridae; the SW lagoon had the lowest densities and biomasses of Acanthuridae and the lowest biomass of Scaridae; the North lagoon had the highest densities of Scaridae and Siganidae and the highest biomass of Acanthuridae and Scaridae. Within the North lagoon, the north part had the highest biomass for all three families, but the highest densities only for Acanthuridae, thus suggesting large fish in that area.

Table II. - Species richness (RS = species/transect), density (D = fish/m<sup>2</sup>) and biomass (B = g/m<sup>2</sup>) for three regions: Ouvea, SW Lagoon and North Lagoon and three subregions of the North Lagoon: East, North and West.

	Ouvea	SW lagoon	North lagoon	North lagoon subregions			Average
				East	North	West	
<b>Species richness</b>							
Acanthuridae	5.76	5.28	4.87	4.50	6.02	4.08	5.30
Scaridae	5.20	5.49	6.34	5.89	7.51	5.63	5.68
Siganidae	0.90	1.95	1.54	1.76	1.79	1.08	1.47
<b>Density</b>							
Acanthuridae	0.146	0.059	0.0124	0.087	0.184	0.101	0.110
Scaridae	0.071	0.108	0.0154	0.124	0.151	0.187	0.111
Siganidae	0.010	0.028	0.0033	0.050	0.033	0.016	0.024
<b>Biomass</b>							
Acanthuridae	21.09	6.51	30.87	10.49	62.92	19.19	19.49
Scaridae	44.35	21.54	72.54	44.54	113.30	59.78	46.14
Siganidae	0.89	4.10	2.65	2.55	3.10	2.29	2.54

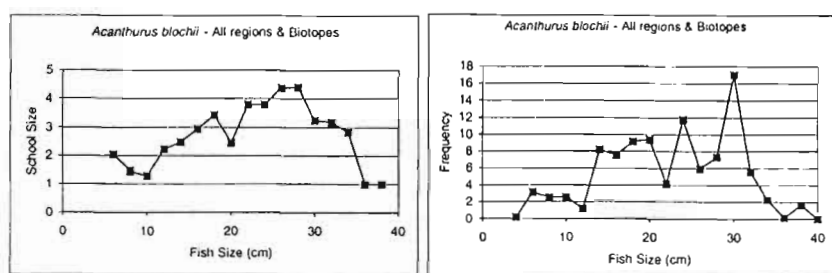


Fig. 2. - School size and size distribution of *Acanthurus blochii*, for the three selected regions and all biotopes combined.

Differences between biotopes for species richness are illustrated in table III. There is a decrease in species richness from the barrier reef towards the fringing reefs for Acanthuridae and Scaridae whilst the opposite trend is observed for Siganidae. Scaridae are however an exception, their species richness increasing from the barrier reef towards the fringing reef in the northern part of the North lagoon. The same trends were found for densities and biomasses which are not illustrated here.

The five major species of these three families can be analyzed separately (Table IV). Ouvea supported the largest densities and biomasses of *Acanthurus blochii*, the SW lagoon had the highest values for *Siganus argenteus* and the North lagoon had the highest densities and biomasses for the three other species. Variations within the north region were important (Table IV) but they confirmed observations between regions, in particular the higher densities and biomasses of *Naso unicornis*, *Scarus ghobban* and *S. microrhinos* in the North lagoon.

FISHEYE will give information on a number of biological and ecological traits. Two examples of these traits, fish size and school size for the five major commercial herbivorous species of New Caledonia are illustrated hereafter.

Size distribution and school size for all regions and biotopes are given for *Acanthurus blochii* (Fig. 2). The frequency of this species tended to increase from fish sizes of 13 cm till 30 cm, then numbers declined rapidly. Similarly the largest schools of *A. blochii* were found for fish sizes between 23 and 28 cm then school size declined with fish size. It is possible to investigate the variations in size distribution or school size of a species between biotopes and regions. This is illustrated also for *A. blochii* (Fig. 3). One notices that the size of this species increased from the fringing reefs towards the barrier reefs for all regions and that there were modes in the size distributions which were common to all regions. The increase in school size with fish size for this species was essentially due to the large schools found in Ouvea (Fig. 3).

Differences between species may also be of interest. This is illustrated for variations in school size and size distribution for the other four major herbivorous species on the barrier reefs of the three regions investigated. Without going into a detailed analysis of these results (Fig. 4), one notices that size distributions for these four species were very close between the SW lagoon and the North lagoon, but differ from those of Ouvea. For instance, larger *Scarus ghobban*, smaller *Siganus argenteus*, and no large *Naso unicornis* were observed in Ouvea. The analysis of school size indicates that the largest schools were formed by *S. argenteus*. For this species, peaks in school size correspond to peaks in size distribution, thus suggesting that the peaks of size distribution could corre-

Table III. - Species richness (number of species/station) of Acanthuridae, Scaridae and Siganidae for three different reef biotopes, three regions and three subregions.

	Ouvea	SW lagoon	North lagoon	North lagoon subregions		
				East	North	West
<b>Acanthuridae</b>						
Barrier	9.40	5.94	5.75	5.67	6.70	4.87
Intermediate		5.49	4.53	4.31	5.69	3.60
Fringing	2.12	4.40	4.32	3.51	5.66	3.78
<b>Scaridae</b>						
Barrier	9.15	6.44	7.16	7.18	6.63	7.66
Intermediate		5.98	6.40	6.32	7.88	4.99
Fringing	1.25	4.06	5.48	4.18	8.02	4.23
<b>Siganidae</b>						
Barrier	1.42	1.13	0.97	1.04	1.08	0.80
Intermediate		2.40	1.89	2.36	2.25	1.07
Fringing	0.38	2.33	1.76	1.88	2.03	1.38

Table IV. - Biomass ( $B = g/m^2$ ), density ( $D = fish/m^2$ ) and number of fish observed ( $N$ ) for three regions: Ouvea, SW Lagoon and North Lagoon and three subregions of the North Lagoon: East, North and West.

	Ouvea	SW lagoon	North lagoon	North lagoon subregions			All regions
				East	North	West	
<b><i>Acanthurus blochii</i></b>							
Biomass	8.51	1.40	3.77	0.67	7.00	3.63	4.56
Density	0.012	0.005	0.008	0.003	0.015	0.007	0.008
Number	1513	2436	2304	390	1217	697	6253
<b><i>Naso unicornis</i></b>							
Biomass	1.25	1.73	8.71	3.66	17.08	5.38	3.90
Density	0.002	0.003	0.008	0.005	0.013	0.005	0.004
Number	261	1247	3194	1247	1296	651	4702
<b><i>Scarus microrhinos</i></b>							
Biomass	5.60	1.308	10.94	5.77	17.84	9.20	5.95
Density	0.0025	0.0014	0.0062	0.0048	0.0089	0.0048	0.003
Number	302	605	2373	940	761	672	3280
<b><i>Scarus ghobban</i></b>							
Biomass	1.60	1.32	5.21	2.31	5.98	7.35	2.71
Density	0.001	0.003	0.013	0.008	0.009	0.021	0.006
Number	141	1248	3825	1201	702	1922	5214
<b><i>Siganus argenteus</i></b>							
Biomass	0.84	2.68	1.25	0.61	2.60	0.54	1.59
Density	0.0075	0.0126	0.0040	0.0018	0.0068	0.0033	0.008
Number	893	5787	1363	451	487	425	8043

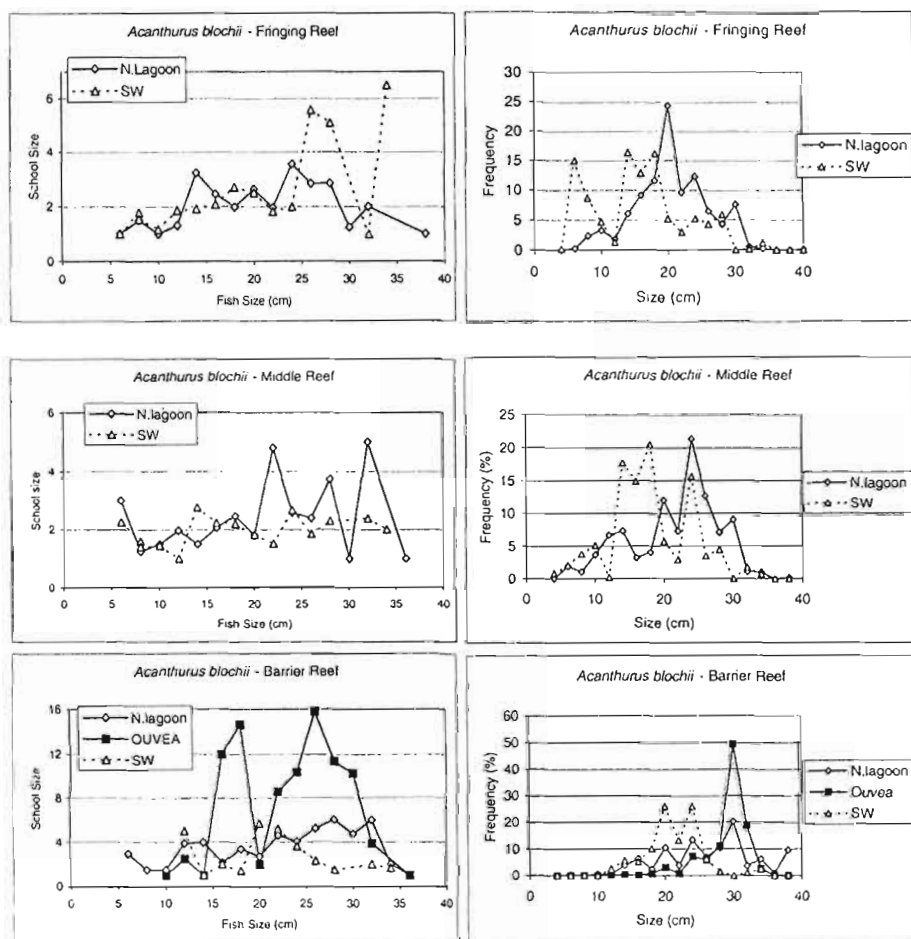


Fig. 3. - School size and size distribution of *Acanthurus blochii* for each region and each biotope.

Table V. - Trophic structure of two regions. All numbers are percentages per column. (RS = species richness, D = density, B = biomass).

	Species richness		Density		Biomass	
	Ouvea	SW Ouvea	Ouvea	SW Ouvea	Ouvea	SW Ouvea
Piscivores	19.30	20.13	4.30	3.61	23.48	11.50
Macrocarivores	30.67	30.76	15.82	5.64	21.27	21.93
Microcarivores	12.24	12.5	10.85	16.03	2.81	4.42
Zooplanktivores	16.25	16.23	48.08	58.38	9.02	14.66
Other planktivores	0.34	1.08	0.33	3.94	0.11	0.49
Macroherbivores	2.67	2.27	1.27	0.69	11.97	4.71
Microherbivores	12.84	11.90	12.44	6.62	26.86	26.09
Coral feeders	4.22	3.66	3.85	2.89	2.75	14.48
Detritus feeders	1.51	1.47	3.06	2.20	1.72	1.72



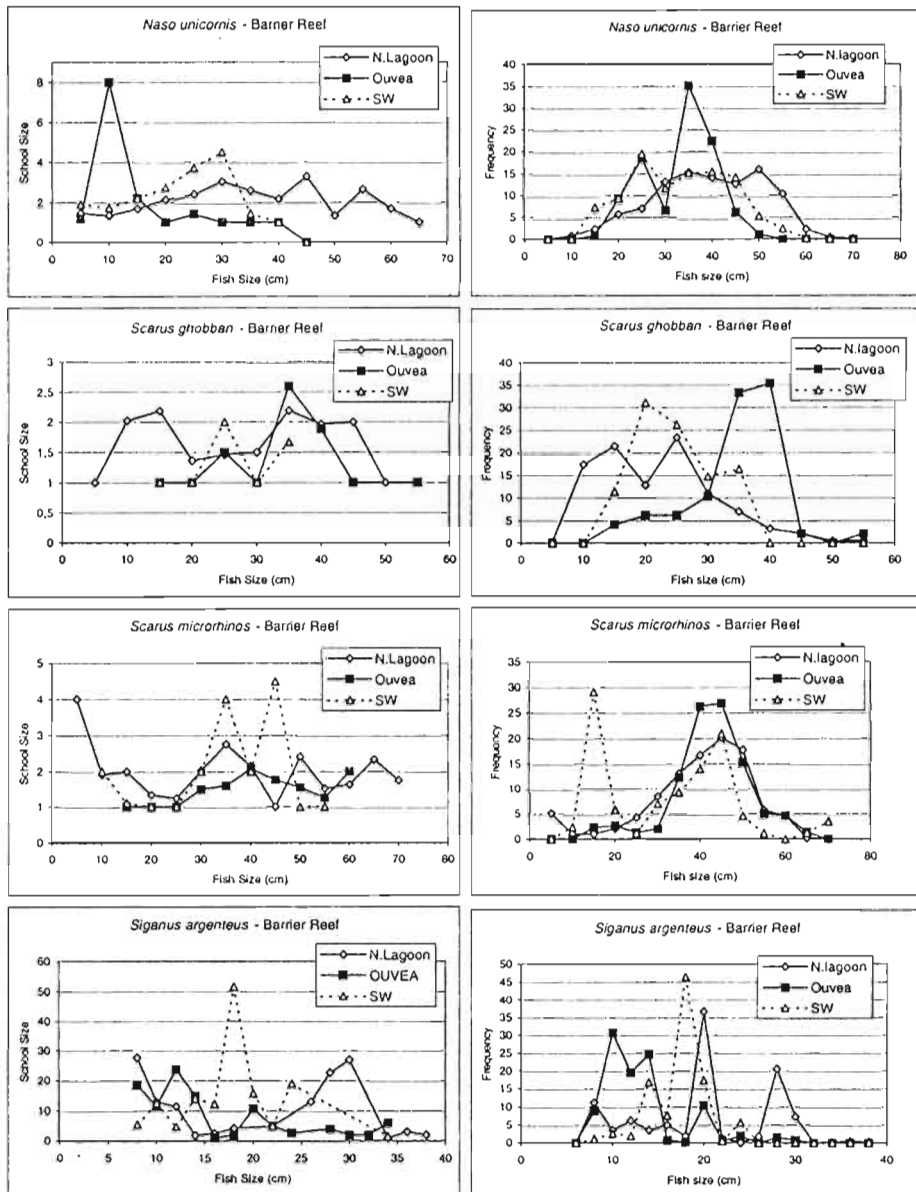


Fig. 4. - School size and size distribution of *Naso unicornis*, *Scarus ghobban*, *S. microrhinos* and *Siganus argenteus* on the Barrier reef of each region.

spond to cohorts. For the remaining three species, school size was small and its variations with fish size did not reflect any particular trends.

Finally, it may be interesting to investigate the importance of herbivores in the trophic structures of reef fish communities. These structures are given for two regions

(Table V) where total counts were available (other regions had only UVCs for commercial species). Species richness of any trophic group hardly changed between the two regions. However, there were important changes between regions for density and biomass of the various trophic groups. Looking at herbivores, both micro- and macro-herbivores made larger contributions to density and biomass in Ouvea than in the SW lagoon. This was compensated by larger contributions of microcarnivores and plankton feeders in the SW lagoon.

## DISCUSSION

### FISHEYE

The development of Internet and the use of data bases through this system is a source of many questions as indicated by an increasing number of workshops and seminars on this matter. Some of the issues are examined here in relation to FISHEYE. Data from a base should be proofed in some way. In the case of FISHEYE no data is preprocessed therefore errors will come mainly at the input of the data into the system. To avoid this problem data are entered twice from two different input sources and the two data sets are overlaid in order to find errors. Of more concern is the precision of the data in the base. For instance, all the length estimates from underwater visual censuses are subjected to an error linked to the observer. In addition, these length estimates are clustered in size classes of increasing size as fish size increases as recommended by Bell *et al.* (1985). Some results may therefore be difficult to interpret. In the present article, the size distributions show a number of modes which may simply be attributed to the lumping by observers of fish sizes into fixed categories. Another problem with data interpretation is that the experimental design is not accessible to the user. In other words, the number of stations in a given biotope is preset and the user can not select a sub-set of these stations in order to build a suitable experimental design to answer his question(s). Thus in the present example, the number of stations for each biotope was not equal and the variations through time could not be taken into account (all the sampling did not take place the same season or the same year). With work covering a large geographical zone and long periods, there may be nomenclature errors (Bailly *et al.*, 1995). To avoid this, the list of scientific names was run through the specialised spell-checking and synonyms programme developed by Froese (1997).

At the moment the data in FISHEYE is also stored in paper files and much of it is not yet in official technical or scientific reports. To write such reports would probably take years because the amount of data in FISHEYE is important. Electronic storage of data is not considered as permanent and citation of such sources in scientific work is still a problem. In order to minimize this problem we are considering the creation of a yearly version of FISHEYE on CDROM disks. Another important issue is the protection against abusive use of the data. The intent of this data base is to make available data which otherwise would not be easily accessible. By letting these data available to anyone there is a risk that some of it may be used in distorted ways or used without prior consent of the data collectors. However, we feel that the benefits to the scientific community far outweigh these risks. In addition it is not possible for the user to gain directly access to the raw data. Therefore, if someone is interested in solving a particular question and needs access to more detailed data he has to contact the collectors of these data. Another protective mechanism is the absence of a measure of data variability in the outputs. This is a double

edge decision, because in many cases a measure of error is necessary to test an hypothesis. We feel that the investigator who needs this type of information will contact us and consequently we should then be in a position to know how the data is used.

One of the major advantage of FISHEYE over a bibliographical approach is that the combination of outputs has no limits. The cost to the user and the time required for retrieving the information are also an attractive part of this approach. In the example illustrated in this article only a low number of the possible outputs of FISHEYE were investigated. In particular, all the information available on the biology of the species (reproduction, diet) were not used, and much of the ecological information were not investigated in detail (habitat type, distribution with depth, etc.).

FISHEYE is still in its initial stages of development. Among the major improvements planed are the possibility to study time series and "horizontal" questioning. At the moment the user has to define the fish species or group of species for which he wishes to retrieve information. This can be viewed as "vertical" access to the data. The user may also wish to retrieve information only according to a variable which is not directly linked to the choice of a species or group of species. For instance, he may want to know all the species found to feed on crustaceans or those which reproduce in the deeper parts of the lagoons during the winter months. This would be "horizontal" access to the data. Another improvement will be the possibility to get some information on the variability of the data.

We plan also to develop other tools within FISHEYE, for instance the possibility to have some information on the productivity of lagoon fish communities. This would allow direct input in softwares such as ECOPATH or ECOSIM. In the future, individual files for each fish species will be built, with a part of fixed information and a part of user interactive information.

Less than half of all the data available at the moment is accessible. The entire data set should be available by mid 1999 and from then on other data sets will be eventually included.

FISHEYE is at the moment available only on one web site, Nouméa. The problem is that this site is only accessible at present through paths with slow data outputs. This may be a problem when using FISHEYE from a remote area. Two solutions are underway. First the data flow from the site of Nouméa will be improved. Second, a mirror site is planed in Paris. This should increase the ease of access to the information considerably. There are more applications of FISHEYE. In this article a direct scientific use of FISHEYE was illustrated. Similar uses and applications for managers, fishermen and education exist.

### **Herbivores from New Caledonian Reefs**

The work presented here can be compared to very similar studies by Russ (1984a, 1984b) or by Letourneur *et al.* (1997a). In some cases (for instance the details on the size distribution or school size), the present results go beyond the type of results presented by these authors. FISHEYE allows to investigate many more traits which could be useful in this kind of research such as the variations of mean size with depth, the variations in habitat type, or the variations of some biological traits such as size at maturity with biotope or depth.

The observed cross shelf distribution of the three families is similar to the findings of Russ on the Great Barrier Reef (1984a, 1984b). These results also confirm observations made in Tonga (Matoto *et al.*, 1997), the Chesterfield (Kulbicki *et al.*, 1989) and

French Polynesia (Galzin, 1985) that Acanthuridae and Scaridae tend to increase with oceanic influence.

Many of the inter-regional variations (for fish size, school size, density or biomass) observed for a given family can be linked to two factors, geography and fishing pressure. Indeed, the SW lagoon lies in waters cooler than the two other regions and is exposed to far more intense fishing activities. Ouvea, where fishing pressure is low, is an atoll and oceanic influence there is strong. The three subregions of the North lagoon are subjected to different terrestrial inputs; the Northern part receives little and the Eastern part a lot. There are also differences in fishing pressure among these three subregions, the Western part gets the highest and the Northern the lowest pressure. Thus, the lowest densities and biomasses of Scaridae and Acanthuridae found in the SW lagoon are probably the result of higher fishing pressure. This is reflected by the smaller size of the fish in that region. The effect of fishing pressure is well illustrated by the differences in density and biomass for all major species within the North lagoon. This is a confirmation of the study by Labrosse *et al.* (1997b).

The variations in trophic structure between the SW lagoon and Ouvea atoll confirm the findings of Kulbicki (1997a) who compared the trophic structure of the barrier reef fish communities of these two locations. The reason why herbivores are less important in the SW lagoon are not known, but resource availability could be a factor. Indeed, Ouvea atoll is essentially autotrophic (Clavier *et al.*, 1992; Kulbicki, 1995), whereas the SW lagoon is heterotrophic (Clavier *et al.*, 1994). In other words, the primary production in Ouvea exceeds the consumption, whereas in the SW lagoon the primary production is supported by external inputs, essentially terrigenous ones. As a consequence algal production per unit of consumer is higher in Ouvea than in the SW lagoon thus allowing a larger percentage of such consumers.

**Acknowledgements.** - We would like to thank all participants of the FISHEYE workgroup, and particularly Michel Ménézo, Philippe Deverge, Marika Tortelier, Yves Penvern, Pierre Laboute and Jacqueline Thomas. We are also grateful to the Northern Province of New Caledonia for permitting the development of FISHEYE.

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## BIOGEOGRAPHY AND DATABASE OF MARINE FISHES IN TAIWAN WATERS

by

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**ABSTRACT.** - The total number of fishes in Taiwan consists of 2,331 species in 233 families. Most of these are marine fish, totaling 2,245 species. Their distribution in Taiwan waters have been recorded in two databases and can be accessed on the internet at [<http://fishdb.sinica.edu.tw>]. The databases were constructed using the GRASS and INFORMIX ONLINE softwares with dbSQL interface. Users can check the actual distribution areas of each species on the map (in a 10' grid system) or obtain a species list for each grid. Faunistic similarity measures and clustering results have shown that the distribution pattern of Taiwanese fishes are mainly affected by temperature, i.e., ocean current (warm Kuroshio coming up from south and cool China coastal waters come down from north) and substratum. Fish faunas are quite different between the north and south, especially among dominant reef species. The overlapping areas are in Penghu on the west and in north-eastern Taiwan on the east.

**RÉSUMÉ.** - Biogéographie et base de données des poissons marins de Taiwan.

A ce jour, 2 331 espèces de poissons, appartenant à 233 familles, ont été recensées dans les eaux taiwanaises, dont 2 245 sont marines. Leur distribution a été enregistrée dans deux bases de données qui sont accessibles par Internet sur le site [<http://fishdb.sinica.edu.tw>]. Ces bases de données ont été élaborées avec les logiciels GRASS et INFORMIX ONLINE, et les interfaces dbSQL. Les utilisateurs peuvent consulter la distribution géographique de chaque espèce sur des cartes (établies avec un système de quadrillage de 10') ou bien obtenir une liste des espèces pour chaque carré de la grille. Des mesures de similitude faunistique et les résultats d'analyses hiérarchiques montrent que la distribution des poissons de Taiwan est principalement fonction de la température, i.e., des courants océaniques (les eaux chaudes du Kuroshio venant du sud et les eaux froides côtières chinoises venant du nord) et du substrat. Les faunes ichtyologiques du nord et du sud sont bien différentes, notamment en ce qui concerne les espèces récifales dominantes. Les zones de recouvrement sont au Penghu, à l'ouest et au nord-est de la partie orientale de Taiwan.

**Key-words.** - Ichtyofauna, Taiwan, Database, Biogeography.

The island of Taiwan has abundant fishery resources. Shen *et al.* (1993) listed 237 families and 2,028 species of fish and estimated more than 2,500 species, altogether. The senior author was granted by the National Science Council a five-year project during 1989-1994 to do a faunistic survey in each region, every year. The data were mostly collected by visual census and specimen collections via scuba diving, including Kenting National Park, Orchid Is., Green Is., Hsialuichiu, Penghu, the reef zone from Chinsan in the northern coast to Maoao in the north-eastern coast, and the rocky substratum along eastern and western coast of Taiwan. For non-reef species, the data were collected from the

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harvests of near shore fisheries including long-liner, bottom trawler or set-net, impingement sample of nuclear power plants, and fish anglers. The fish specimens deposited at several museums or research institutes were also verified and included in the list. So far, only three papers have been published on these areas: Hsiaoliuchiu with 608 species (Chen *et al.*, 1992), western Taiwan with 664 species (Shao *et al.*, 1993), and Penghu with other 648 species (Shao *et al.*, 1994). Other species numbers are 663 (north), 332 (north-eastern), 715 (eastern), 601 (Green Is.), 558 (Orchid Is.) and 1,104 (South, i.e., Kenting). Most of these fish are coral reef fishes. Additionally, the authors updated the species number to 392 at Tung-Sha (Pratas Is.) (Chen *et al.*, 1995) and to 421 at Nansha (Spratly Is.) (Chen *et al.*, 1997).

#### Additions to the Taiwanese fish fauna after 1994

After the book of "Fishes of Taiwan" (Shen *et al.*, 1993) was published, we have reported many new additions of fishes to the Taiwanese fish fauna. To date, the following new species were added: *Pseudochromis striatus* Gill *et al.*, 1994; *Schismatogobius ampluvinculus* Chen *et al.* 1995; *Pseudocoris ocellatus* Chen & Shao, 1995; *Cirrimaxilla formosa* Chen & Shao, 1995; *Gymnothorax niphostigmus* Chen *et al.*, 1996; *Enneapterygius cheni* Wang *et al.*, 1996; and 3 new species of *Rhinogobius* (Chen and Shao, 1996). Another new species of *Gymnapogon* and one species of *Callogobius* are in preparation. As to the Taiwanese new records of fishes since 1994, the following fishes were reported: 12 species of Muraenidae (Chen *et al.*, 1994); 1 species of newly recorded family (Microdesmidae), *Gunnellichthys curiosus* (Chen *et al.*, 1994); 5 species collected from western coast (Lin *et al.*, 1994); 4 species of Labridae (Wang *et al.*, 1994); 2 species of the genus *Asterorhombus* from the Spratly Island (Lin *et al.*, 1995); 5 species of the genus *Diaphus* (Kao and Shao, 1996); 12 species of gobies living in coral reefs (Chen *et al.*, 1997); 5 species of Carangidae (Lin and Shao, 1998), and 7 species of *Amblyeleotris* (Chen *et al.*, 1998).

Other new species or new records published by Taiwanese fish taxonomists since 1994 include 5 new species of Myxinidae (Kuo and Mok, 1994; Kuo *et al.*, 1994); 5 new species of Tripterygiidae (Shen and Wu, 1994); 2 new species of *Rhinogobius* (Aonuma and Chen, 1996); 1 new species of Synphobranchidae, *Dysomma opisthoproctus* (Chen and Mok, 1995); 1 new species of Congridae, *Ariosoma nancyae* (Shen, 1998); and 1 new record of a moray eel (Chen and Böhlke, 1996); 2 new records of gobies (Chen *et al.*, 1995), and a long-nosed chimaera (Shao and Huang, 1997).

#### Biogeographical distribution of marine fishes in Taiwan

The updated checklist of fish species in Taiwan contains a total number of 233 families and 2,331 species. About three-fifth among them are coral reef fishes, i.e., 72 families and 1,425 species. The highest diversity region is in Kenting, then east, north, west, Penghu, Green Is., Hsiaoliuchiu, and Orchid Is.

Chaetodontidae, Scaridae and Acanthuridae are common in the south, Hsiaoliuchiu, Orchid Is. and Green Is. However, these reef fishes are rare in the north, Penghu and western Taiwan, where the corals are not so prosperous. The geographical distribution of reef fishes in Taiwan is highly correlated with the distribution of corals.

Table I lists the species numbers of 21 families of important reef fishes occurring in Taiwan. Four categories, i.e., abundant (A), common (C), occasional (O), and rare (R) were used to represent their abundance based on diving observation. The results show that the majority (56%) of reef fishes is rare, i.e., only one or two individuals could be found during one year survey on the basis of about 20 diving hours in average; 27% are occa-



Table I. - Abundance of exotic marine fish species in the waters around Taiwan. Asterisks (\*) represent edible or economical species.

Family (common names)	Total species in the world	Total species in Taiwan	Percentage in the world	Current species numbers in Taiwan			
				Abundant	Common	Occasional	Rare
Acanthuridae (surgeonfishes) *	72	40	55.6	3	16	5	16
Apogonidae (cardinalfishes)	207	80	38.6	2	12	16	50
Balistidae (triggerfishes)	40	18	45.0	0	3	3	12
Chaetodontidae (butterflyfishes)	114	46	40.4	3	0	10	33
Cirrhitidae (hawkfishes)	32	10	31.3	2	1	3	4
Gobiidae (gobies)	#2000	195	9.8	1	21	66	107
Holocentridae (squirrel-fishes) *	65	28	43.1	0	6	9	13
Labridae (wrasses)	>500	127	25.4	14	28	39	46
Lutjanidae (snappers)	97	48	49.5	3	10	12	23
Monacanthidae (filefishes)	95	23	24.2	2	2	8	11
Muraenidae (moray eels) *	200	51	25.5	0	3	9	39
Ostraciidae (boxfishes)	33	10	30.3	0	2	1	7
Pomacanthidae (angelfishes)	74	31	41.9	0	2	7	22
Pomacentridae (damselfishes)	315	101	32.1	10	23	20	58
Pseudochromidae (dottybacks)	98	13	13.3	1	1	6	5
Scaridae (parrot fishes) *	83	28	33.7	0	8	16	4
Serranidae (groupers) *	449	116	25.8	2	6	21	88
Siganidae (rabbitfishes) *	27	10	37.0	0	1	6	3
Solenostomidae (ghost pipefishes)	3	2	66.7	0	0	0	2
Syngnathidae (pipefishes)	215	29	13.5	0	0	3	26
Tetraodontidae (puffers)	121	29	24.0	0	3	14	12
Total	4229	1035		43	150	278	581
(%)			24.5	4.2	14.5	26.9	56.1

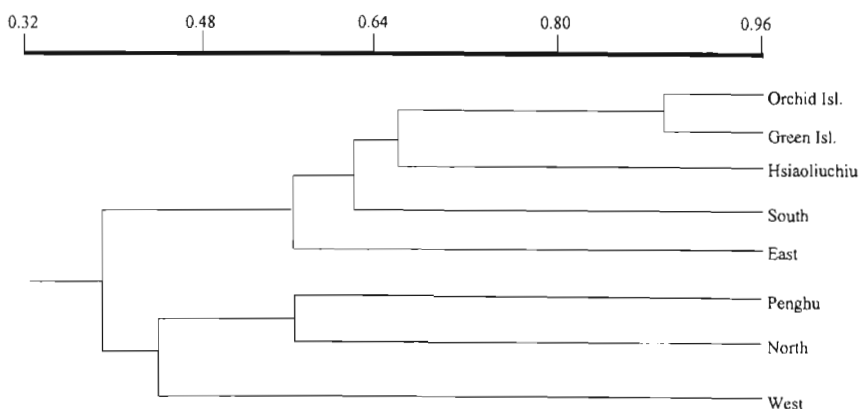


Fig. 1. - UPGMA phenogram showing the faunistic relationships of fish communities among eight different regions in the waters around Taiwan.

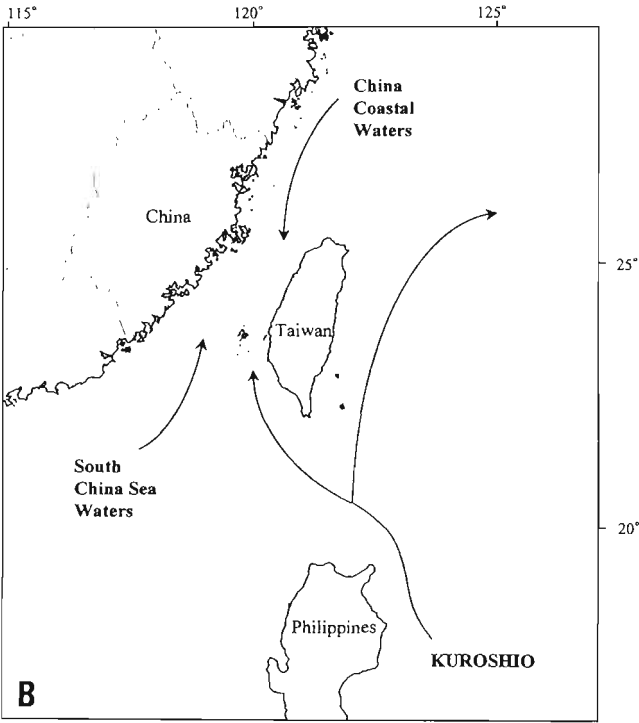
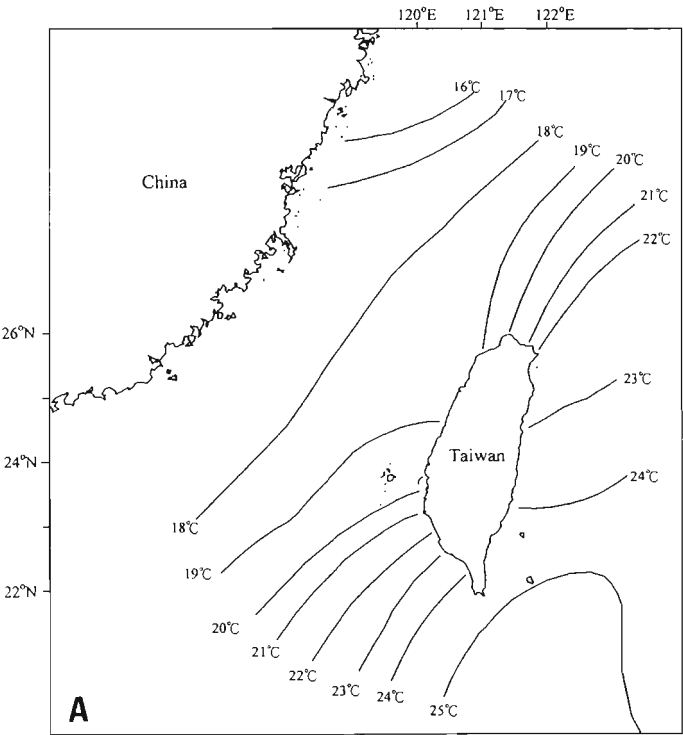
sional species, with a few individuals or occurrences 2-3 times per year. The common and abundant species comprise only 14.5% and 4.2% respectively. Thus, although the total number of important coral reef fish species in Taiwan is almost one-quarter (24.5%) of those in the world, the number of individuals is quite low.

Two large distinct groups could be recognized from UPGMA clustering result (Fig. 1). One contains eastern and southern Taiwan, Hsiaoliuchiu, Orchid and Green Is. The other contains Penghu, northern and western Taiwan. This pattern coincides with that of the ocean currents and surface temperature (Fig. 2), modified by substratum types. In other words, two major zoogeographical distribution zones in the waters around Taiwan could be distinguished:

#### *Kuroshio affected zone*

The main current and its branch come from south of the equator up to the southern tip of Taiwan. The strong main current flows to the east coast passing through the islets of Orchid and Green Is. A weak side-branch flows through the Taiwan Strait up to Hsiaoliuchiu and south Penghu. In winter it flows along the Penghu Channel or even penetrates into more northern parts. In summer it flows along the western shoreline, crossing the Tropic of Cancer. This zone is the major area of coral reef fishes in Taiwan. Some of them, at least 10 species, are distributed here as their northernmost boundary, such as *Samariscus triocellatus*, *Sillago chondropus*, *Thysanophrys otaitensis*, *Pseudoplesiops knighti*, *Apogon taeniophorus*, *Scolopsis trilineatus*, *Paracheilinus carpenteri*, *Canthigaster solandri* and *Siganus javus*. Eastern Taiwan, because of its strong currents and deep waters contains mostly deep water or oceanic meso-pelagic fish species.

Fig. 2. - Distribution pattern of sea water temperature around Taiwan (A), this surface temperature pattern is actually determined by the northward warmer Kuroshio current and South China Sea Waters, as well as the southward colder China Coastal Waters (B).



### *Southwestern monsoon affected zone*

In the summer season, the southwesterly summer monsoon drives the South China Sea surface waters into Taiwan Strait through the Penghu Channel. These lighter surface waters can flow over the Changyuen ridge and then along the west coast of Taiwan to the East China Sea. However, the flows change during the northeasterly winter monsoon, which drives the colder and fresher China coastal waters into the southern Taiwan Strait (Jan, 1995). In other words, the warmer and more brackish waters of the South China Sea are retarded by the wind stresses and turned northwestward at south of the Changyuen ridge during the winter. Some reef fishes originating from the South China Sea could be transported to Penghu, northern or western Taiwan from this southwestern monsoon current. However, the low water temperature of the China coastal waters will inhibit the distribution of more tropical reef fish species so that the number of reef species in this zone is less than that of the Kuroshio affected zone. Penghu Islands at the Tropic of Cancer is the boundary or overlapping area between these two zoogeographical zones. Thus, the number of coral reef fish species is higher in southern Penghu than northern Penghu and northern Taiwan. Besides those non-coral fishes such as Sciaenidae or Cheilodactylidae, which are associated with China coastal waters, there are some coral reef fishes in this region that have not yet been found in Japan, such as *Halichoeres dussumeri*, *H. timorensis*, *Apogon holotaenia*, *A. nigripinnis*, *Valenciennesa immaculata*, *Parascorpaena picta* and *Epinephelus bleekeri*. Except for *H. timorensis*, all are common species in this area but are not found in other areas of Taiwan. It is believed that these species are originally Indian Ocean species that expanded to the western Pacific.

Other similar species which have been reported from Japan, but not yet been discovered in the Kuroshio affected zones include: *Ecsenius lineatus*, *E. namiyei*, *Upeneus moluccensis*, *Chromis cinerascens* and *Parapercis snyderi*. Finally, the most distinctive fish species composition was on the western coast, which was mainly due to different types of substratum and topography, i.e., shallow and sandy or muddy habitat.

### **Fish database of Taiwan and its access on the Web**

In Taiwan, only curatorial databases of fish at both Academia Sinica (AS) and National Taiwan University (NTU), existed previously. The specimens deposited at other museums have not yet been computerized. The only distributional database of marine organisms in Taiwan was established by the authors at AS. This fish database was established by using ACCESS 7.0 and now can be accessed on the Web at [<http://fishdb.sinica.edu.tw>]. This database is briefly introduced below.

There are three distributional databases: Inshore economic fishes, coastal fishes, and freshwater and estuary fishes. They were constructed using GRASS (Geographic Resources Analysis and Support System) and INFORMIX ONLINE with dbSQL interface. Users can easily check the actual distribution areas of each fish species on the map or get a species list for each 10' grid of coastal waters around Taiwan. The database contains a fish's scientific name, Chinese names, both English and Chinese common names, localities, seasons (months), fishery methods, and abundance data. Four categories of abundance (abundant, common, occasional or rare) for each month can be plotted in different colors on a map for each fish species. There are specimen photos and detailed descriptions (in Chinese language) of each species including morphological character, ecological habitats, and geographical distribution.

Original fish data were collected region by region every year. For inshore economic fishes, the project was supported by the Council of Agriculture in order to promote

the utilization, management, and conservation of coastal fishery resources in Taiwan. For coastal marine fishes of Taiwan, the project was supported by the NSC. The basic differences of this coastal fish database from a previous inshore economical one is that this database includes non-economical smaller fishes, such as coral reef fishes. All data of coastal fishes were recorded from field work, not indirectly from fishermen's questionnaires.

We are planing to put the most updated fish checklist and the reason (citation) for each change (new addition, deletion or name change) on the Web to provide the most updated fish taxonomic information in Taiwan. The most updated erratum of "Fishes of Taiwan" (Shen *et al.*, 1993) was provided in this database as well to serve all users in the world.

Actively provide our local fish faunistic data in order to merge Taiwanese data into some global databases is very important because users can easily obtain updated fish information of Taiwan through, e.g., the global FishBase (CD-ROM) (Froese and Pauly, 1996). There are several different approaches to export our country data to FishBase: file transfer, forms, reprints or unpublished data, and photos. Species 2000, a project for indexing the world's known species on the internet, also links to FishBase for taxonomic information. Finally, we hope that our experiences of building a fish database in Taiwan could help fish taxonomists in other Asian-Oceanic countries to build up similar databases. Ultimately, everyone should be able to get the most update fish distribution data from all of the world through the internet.

**Acknowledgements.** - We thank Dr. Chien-Ming Ho, Ms Yu-Mei Shao at Institute of Information Engineering, Academia Sinica for their technical support on developing the GIS system. Dr. I.S. Chen, H.M. Chen and several of my former students helped in field and technical works including database establishment, especially C.Y. Wu, Y.Y. Wu, L.Y. Hsieh and S.H. Lee. This study was mainly supported by National Science Council and Council of Agriculture to the senior author.

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### **Workshop 3**

## **FUTURE OF ICHTHYOLOGICAL RESEARCH IN THE TROPICAL INDO-PACIFIC**

Chairperson: **Kent E. CARPENTER**

CARPENTER K.E. & J.R. PAXTON. - The future of systematic ichthyological research in the tropical Indo-Pacific. 683-693

*Le futur de la recherche en Ichtyologie systématique dans l'Indo-Pacifique tropical.*





## THE FUTURE OF SYSTEMATIC ICHTHYOLOGICAL RESEARCH IN THE TROPICAL INDO-PACIFIC

by

Kent E. CARPENTER (1) & John R. PAXTON (2)

**ABSTRACT.** - Many areas of systematic ichthyological research remain wide open for investigation in the Indo-Pacific. Undescribed species and other valuable material are expected from relatively unexplored marine and freshwater habitats and regions. For example, from the marine realm deep reef, outer shelf, and deep sea habitats have been sparsely collected with mostly inadequate gear. Many lakes and rivers in the region have never been visited by an ichthyologist. Certain areas such as Sumatra, where rich freshwater fish faunas are expected, require extensive additional collections. The freshwater fish fauna of Myanmar needs serious critical review. In addition to habitats and regions, certain groups of fishes from the region remain highly problematic taxonomically. For example, most marine anguilliform and many cypriniform groups require extensive revisionary work. The complex and often unresolved geological history of the region, particularly around Southeast Asia, continues to confound both marine and freshwater biogeographic hypotheses. Only a handful of phylogenetic hypotheses down to species level are available among the numerous fish families, leaving biogeographic models largely untested and supraspecific classification less defensible. New collecting and analytical methods, infusions of scientific support, and plenty of dedicated research time are required to make significant research achievements. Many of these research gains are urgent, in view of the increasing degradation of both marine and freshwater habitats of the Indo-Pacific.

**RÉSUMÉ.** - Le futur de la recherche en Ichtyologie systématique dans l'Indo-Pacifique tropical.

La région indo-pacifique reste un vaste champ d'investigation pour la recherche en Ichtyologie systématique. Les zones marines et les eaux douces, encore mal explorées, dissimulent probablement de nombreuses espèces à découvrir. Certaines zones marines, comme par exemple la pente récifale externe, le plateau continental externe ou encore les habitats profonds, ont été peu explorées ou de manière superficielle. Des lacs et des rivières n'ont jamais été prospectés. Les régions de Sumatra et de Myanmar nécessitent une étude plus approfondie, non seulement pour la découverte de nouveaux taxons, mais aussi pour résoudre les énigmes posées par certains groupes. Une révision est nécessaire pour la plupart des Anguilliformes marins et de nombreux Cypriniformes. Les hypothèses biogéographiques concernant les ichtyofaunes marines et d'eau douce restent encore confuses au niveau de l'histoire géologique de la région. Les nombreuses hypothèses phylogénétiques au niveau spécifique ne sont pas suffisantes pour tester les modèles biogéographiques actuels. De nouvelles méthodes de prospection et des recherches plus intensives sont devenues indispensables et urgentes du fait de la dégradation croissante des milieux aquatiques dans toute la zone indo-pacifique.

**Key-words.** - Ichthyology, ISEW, ISW, Indo-Pacific, Systematics.

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The purpose of this paper is to identify promising new avenues of research and document the urgent need for research in certain areas of systematic ichthyology in the tropical Indo-Pacific. Information on the status and future needs of research are taken from the literature and the collective knowledge of participants of a workshop of the same title held during the Fifth Indo-Pacific Fish Conference held in Noumea. This workshop was called for by the organizers of the Conference in recognition of dwindling support for systematic ichthyology in spite of the need to increase this support to meet the global biodiversity preservation challenge. We limit the geographical coverage to the classical interpretation of « tropical Indo-Pacific ». That is, the tropical and subtropical Indian and western and central Pacific Oceans, and South and Southeast Asian, Australian, and Pacific Island freshwater habitats. African freshwater concerns are not addressed. Both marine and freshwater habitats are considered. The most important topics for future research include further exploration, taxonomic work, new approaches to biogeography, phylogenetic hypotheses to species level, and recognition of conservation priorities.

### EXPLORATION

Extensive collections of fish specimens have been made in the tropical Indo-Pacific region but there are many habitats and areas that have not been explored adequately. W.N. Eschmeyer (pers. comm.) estimates from his fish species taxonomy database that there are 25,000 valid species names worldwide and that this number will eventually reach 35,000 species. A high proportion of these new discoveries will come from the Indo-Pacific since it contains the most diverse fish fauna and relative to its size and diversity represents one of the least studied regions of the world.

A large majority of the diversity of fishes is found on coral reefs and these and other rich nearshore marine habitats have been extensively collected. Many of the advances in our knowledge of this diversity in the past few decades have been due to the collection efforts of a number of researchers who know the fauna well and can recognize a novel find. New species continue to be discovered from Indo-Pacific reefs and it is likely that this trend will continue for many years to come. This is particularly true for certain geographic regions where large tracts of coral reefs have never been collected or visited by an ichthyologist. The highest diversity centered in the Indo-Malay-Philippine archipelago (most particularly in the Banda Sea and eastern Indonesia) will continue to yield new species from coral reefs and other nearshore habitats. The Andaman Sea is an area of endemism that has not been collected adequately and much is still to be learned about the biodiversity of this area. The key to understanding the patterns of diversity in the north-west Indian Ocean region lies in a greater understanding of the as yet under collected nearshore fauna of the southern Red Sea, the Gulf of Aden, and through the entire length of Somalia. The coastal fauna of Vietnam and southern China require further exploration. A number of western and central Pacific localities such as Papua New Guinea, the Solomon Islands, and Wallis and Futuna are also still under collected. In addition, it is clear that museum collections are inadequate for numerous nearshore taxa in many areas; any serious collecting effort anywhere across coastal habitats of the tropical Indo-Pacific will undoubtedly yield new discoveries. The coastal habitats that are most in need of exploration are rocky and sandy high surf zones and muddy shallow areas (particularly around mangroves) since these habitats have typically been low priority during most collecting expeditions to the region.

Deep sea exploration of the Indo-Pacific is also far from complete. The deep sea is the largest aquatic habitat in terms of both volume and extent of bottom, extending from 150-200 m depth to 10,900 m at the bottom of the Marianas Trench in the western Pacific, with an average depth of 3,700 m. While the general study of fishes goes back to at least the time of Aristotle, the study of deep sea fishes, except for rarely caught specimens, commenced only 125 years ago with the Challenger Expedition of 1872. Marshall (1954) reviewed the early history of deep sea biology.

In addition to the relatively short period of time during which deep sea fishes have been studied, our knowledge is also limited by our current technology. The deepest fish to date, a cuskeel of the family Ophidiidae, has been trawled at 8,370 m (Cohen and Nielsen, 1978). We assume the deepest 2,500 m of the ocean is depauperate, but have virtually no evidence. We do know that fish life exists in the deepest trench, as a flatfish of unknown identity was seen by Piccard and Walsh on the bottom of the Challenger Deep in the Marianas Trench out of the bathyscaphe Trieste in 1960 (Idyll, 1964).

To forecast adequately future collection needs of the deep sea requires a summary of current collection strengths. A comprehensive survey of present collections is not possible here. Some idea of current coverage of mesopelagic stations in the tropical Indo-Pacific is given in Becker's review of the Myctophidae of the World Ocean (Becker, 1983: fig. 3). This map of primarily Russian stations, with most of the fishes in the Shirshov Institute of Oceanology in Moscow, is noteworthy for the density of stations in the western Pacific. Particularly surprising is the good coverage of the northern Indian Ocean. An indication of historical deeper stations is indicated in Ebeling's review of the bathypelagic genus *Melamphaes* (Ebeling, 1962: fig. 42). Although the western central tropical Pacific is strikingly barren in this 35 year old map, intensive midwater collecting in that area, at least in the upper 1,000 m, has since been undertaken by ORSTOM in Noumea; the fish collections resulting from more than 2,000 midwater trawls await registration at MNHN in Paris. A significant collection of tropical Pacific midwater fishes is held at Scripps Institution of Oceanography in La Jolla, with fishes from more than 5,500 stations from the entire Pacific is available. The number of deep sea collections from around Hawaii also is estimated in the thousands (T. Clarke, pers. comm., Oct. 1997). A smaller, but mostly unworked, collection of 400 stations by Tokai Fisheries from the northwest Pacific is now being sorted and registered at the National Science Museum, Tokyo; some 10% of these midwater trawls sampled depths of 2,000-2,600 m. We are not aware of similar maps that indicate coverage of benthic sampling in the tropical Indo-Pacific. Merrett and Haedrich (1997) have summarized much information about benthic deep sea fishes.

The largest gaps in deep sea collecting of both pelagic and benthic fishes are related to depth. Of the 10,000+ collections of deep sea fishes taken in the tropical Indo-Pacific, we estimate less than 10% have been taken in depths below 2,000 m, and less than 1% in depths below 3,000 m; the deepest 8,000 m in the Indo-Pacific are virtually unsampled, especially in the tropics.

A specific unsampled habitat even in the upper 1,000 m is the rocky benthos, where trawling is difficult to impossible. This includes the deep reef (50-100 m), sea mounts, and other rough bottom habitats on the outer shelf (100-200 m), and continental slope and other zones below 200 m. Limited experimental sampling over rough bottom has yielded a very high percentage of new species in the Caribbean (C.R. Robins, pers. comm.) and the Galapagos (J. McCosker, pers. comm.). Similar efforts will likely yield a high degree of new discoveries in the typical high diversity of the Indo-Pacific. Mixed gas rebreather diving shows promise to sample some of the deep reef habitats and these have

yielded a number of new discoveries in the Indo-Pacific (J.E. Randall, pers. comm.). Improved or novel approaches to sampling over deep rough bottom habitats need to be worked out in order to make this exploration possible. For example, better slurp gun and other collecting techniques from manned submersibles could be developed. The use of remotely operated vehicles or traps and nets that float to the surface and can be located after short periods may be important for sampling over deep rough bottom habitats. Two other techniques may see increased collections from this area in the future. Much commercial orange roughy (*Hoplostethus atlanticus*) fishing targets spawning aggregations over rocky pinnacles or peaks in depths near 1,000 m. Commercial techniques to sample close to the bottom, but avoid fouling the net, should be translatable to research cruises at these and greater depths. The other possible technique involves use of trammel nets, that have recently been successfully set over deep water reefs in northern Australia (Lloyd and Mounsey, 1997). Deep sea gear technology development and operation is likely to be expensive. Substantial support, perhaps through international cooperation will be needed to make this exploration feasible.

The extraordinary commercial concentrations of the deep sea orange roughy, *Hoplostethus atlanticus* of the family Trachichthyidae, were found less than 20 years ago primarily in the southern hemisphere waters of New Zealand and later southern Australia. More recently commercial quantities of a number of species of oreo dories of the family Oreosomatidae have also been discovered. Merrett and Haedrich (1997) describe these fisheries. Perhaps the possibility of further commercial deep sea species will encourage much needed exploratory fishing in even deeper waters.

To increase the number of fish samples below 2,000 m will require research vessels with more wire, and both the money and the will to spend large amounts of time needed for deep sea trawling. Research vessels and money for exploratory research appear to be decreasing rather than increasing at the present time. In Australia the sale of the Royal Australian Navy research vessel « H.M.A.S. Cook » some years ago was followed by the 1997 sale of N.S.W. Fisheries « F.R.V. Kapala ». The CSIRO « F.R.V. Southern Surveyor » does not go to sea full time due to lack of funding.

Country	# spp.	% Complete
Bangladesh	260	90%
Brunei	55	30%
Cambodia	215*	60%
India	748	90%
Indonesia	1300	70%
Laos	262	50%
Malaysia	600	85%
Myanmar	300	50%
Pakistan	159	90%
P.N. Guinea	329	70%
Philippines	330	80%
Singapore	45	100%
Sri Lanka	90	95%
Thailand	690	90%
Vietnam	450	80%

Table I.- Numbers of species (# spp.) of freshwater fishes recorded from Asian countries and estimate of the percent of the complete fauna these species numbers represent.

A number of freshwater habitats and regions of the tropical Indo-Pacific have been inadequately explored. For example, headwaters, rapids, springs, and blackwater habitats such as peat swamps are often difficult and dangerous to reach and have a high rate of endemism. Hundreds of undescribed species will likely be found in these relatively unexplored habitats (Kottelat and Whitten, 1996). The freshwater fish fauna of many geographic areas also are inadequately known. For example, there is only a rudimentary understanding of the faunas of Myanmar (Burma), Brunei, Laos, Cambodia (Table I), and all watersheds of Sumatra, Sulawesi, Kalimantan, and Irian Jaya. The extent of the inadequacy of our knowledge of freshwater fishes from this region is exemplified by the discrepancy in recent estimates of the extent of the fauna from experts. Kottelat and Whitten (1996) list 215 known freshwater species reported from Cambodia and estimate that this represents around 60% of the total fauna. In contrast, Rainboth (1996) lists around 500 species from freshwater habitats and estimates a total of 1200 will eventually be recorded. Many insular faunas of the Pacific and Indian Oceans are also inadequately described. Clearly, a great deal of exploration and taxonomic work will need to be done before an adequate description of the freshwater fish biodiversity can be accomplished.

The future of ichthyological exploration in the tropical Indo-Pacific will rely chiefly on the success of international cooperation. Expeditions that can cover the many less accessible locations will be expensive and some form of international funding cooperation may be necessary. Also, local institutions and researchers must be integrated in expeditions for efficient exploration and to help justify permits and the use of essential collecting techniques such as the use of rotenone.

## TAXONOMY

The basic taxonomy of a large proportion of Indo-Pacific fish families and genera is poorly understood. This can be a serious hindrance to research on these fishes, since the names are used to attach observations to the units of study in biology. Confusion in the identity of the unit of study can invalidate the information communicated to other scientists through the literature.

An estimate of the degree of disarray of the taxonomy of marine fishes in the Indo-Pacific can be gained from a review of the manuscript «FAO Identification Guide for Fishery Purposes: The Living Marine Resources of the Western Central Pacific» (K.E. Carpenter and V. Niem, editors). This guide will include six volumes and the first volumes will be printed in 1998. The area covered includes FAO fishing area 71 and the South Pacific Commission area. In addition to other marine resource groups such as seaweeds, molluscs, and crustaceans, it covers all families of fishes found in the area. Ninety-five authors, representing the world's authorities on their respective taxonomic group, contributed. Of the 232 families of bony fishes included, 85 of these are noted as requiring extensive revision or having a large proportion of species of questionable distribution or taxonomic status. Therefore, around 32% of bony fish families are in serious need of taxonomic revision. This is a minimum number since taxonomic status was not fully noted for many of the deep sea and other groups of minor economic importance. The Anguilliformes was listed as one of the most challenging groups taxonomically. Of the 14 families containing somewhere around 300 species, all but three families were listed as in need of extensive revision. The epipelagic and coastal fishes in most need of revision include most of the Rajiformes, the Clupeidae, Pristigasteridae, Engraulidae, Syngnathi-

dae, Platycephalidae, Scorpaenidae, Triglidae, Acropomatidae, Kuhliidae, Apogonidae, Carangidae, Lethrinidae, Pempheridae, Scatophagidae, Uranoscopidae, Clinidae, Trypterygiidae, Gobiidae, Sphyrnidae, Soleidae, and Cynoglossidae. Deep sea fish groups requiring more study at the alpha taxonomic level include some frustrating gaps in knowledge for dogfish sharks (Squalidae), catsharks (Scyliorhinidae), many rays (Rajiformes), conger eels (Congridae), slickheads (Alepocephalidae), dragonfishes (Stomiidae), greeneyes (Chlorophthalmidae), barracudinas (Paralepididae), morid cods (Moridae), rattails (Macrouridae), cuskeels and brotulas (Ophidiiformes), jellynose fishes (Ateleopodidae), roughies (Trachichthyidae), bigscales (Melamphaidae), whalefishes (Cetomimidae), mosaicscales (Megalomycteridae), macruricyttids (Macruricyttidae), manefishes (Caristiidae), bathyclupeids (Bathyclupeidae), and swallowers (Chiasmodontidae).

The taxonomy of freshwater fishes of the Indo-Pacific is also poorly understood. Nearly all families of freshwater fishes are in serious need of revision (T. Roberts, pers. comm.). Some of the most problematic groups are the most diverse and also of considerable economic importance. These include the Cyprinidae, Bagridae, Channidae, Schilbeidae and Siluridae (C. Ferraris, pers. comm.). Other families in dire need of revision include the Balitoridae, Cobitidae, Mastacembelidae, Sisoridae, and the Ambassidae (C. Ferraris, pers. comm.).

The future of taxonomic research will rely on continued support. Part of this will be driven by biodiversity concerns and resource management needs. Taxonomic clarification is clearly defensible for groups of economic importance since biological management requires correct species identification. Epipelagic groups fit this category. Although epipelagic species make up less than 2% of total species worldwide, their economic importance is disproportionately high. Of the top 69 taxonomic units listed in the FAO Fisheries Statistics Yearbook (table A-1) in terms of total landings, 25 of these are epipelagic taxa. The important families are Engraulidae, Clupeidae, Scomberesocidae, Trichiuridae, and Scombridae. Therefore, studying the systematics and population level phenomenon of these epipelagic species is important for application in resource management and conservation issues.

In addition to continued research on adult fishes, a great deal of work is left to be done for collection, curation, and taxonomy of larval fishes. Collection efforts are particularly needed in coastal areas and hard bottom habitats deeper than 30 m. An understanding of many of the life history parameters that allow sound management of fish populations is locked up in the biology of larval fishes. The first step in unraveling this information must lie in our ability to correctly identify the species. However, much work is still to be done on larval taxonomy at both the generic and specific levels. This work can only be accomplished through collection management support to make existing larval fish collections accessible.

## SYSTEMATICS AND BIOGEOGRAPHY

That evolutionary relationships are poorly understood is not surprising given the many gaps in our knowledge of the basic taxonomy of Indo-Pacific fishes. Some progress has been made in understanding the higher order relationships of both elasmobranchs (Compagno, 1988; Shirai, 1992) and teleosts (Johnson, 1993). However, the monophyly and interrelationships of the largest and most diverse order represented in the Indo-Pacific

(and the most diverse order of vertebrates), the Perciformes, has never been seriously tested. Only a handful of families have phylogenetic hypotheses attempted to species level. These are mostly restricted to the percoid families Teraponidae (Vari, 1978), Caesionidae (Carpenter, 1990), Pseudochromidae (Winterbottom, 1985), and Plesiopidae (Gill, pers. comm.). However, the interrelationships of percoid families and the Percoidei, the largest and most diverse suborder of Perciformes, are almost totally unknown. An understanding of the limits and relationships of percoid families is necessary before the immediate outgroups of a great bulk of the diversity of Indo-Pacific fishes can be hypothesized. Some progress has been made in molecular systematics of fishes (Kocher and Stepien, 1997), but most groups still have not benefited from this approach.

Few cladistic studies have been completed on deep sea fishes. Notable for these studies are the dragonfish order Stomiiformes (Weitzman, 1974; Fink and Weitzman, 1982; Fink, 1985; Harold and Weitzman, 1996), the lizardfish order Aulopiformes (Baldwin and Johnson, 1996), and the squirrelfish orders Beryciformes and Stephanoberyciformes (Moore, 1993; Johnson and Patterson, 1993). An encouraging start has been made on the lanternfish order Myctophiformes (Stiassny, 1996). Clearly most groups of deep sea fishes require much further study of their relationships in the future. Related studies on such topics as DNA sequences are available for few deep sea groups, like the gonostomatid genus *Cyclothone* in an impressive study by Miya and Nishida (1995). A similar study of myctophid genera is currently underway by Yamaguchi (1996).

Many opportunities exist for interpretation of biogeographic pattern in the Indo-Pacific. Recent reconstructions of the geological history of Southeast Asia and the western Pacific (Hall, 1996, 1997) will allow specific tests based on the vicariance approach. For example, the hypothesis that the major island components of the Philippines originated from four widely separated sources (Hall, 1996) could be the key to understanding the diversity and distribution patterns of this area. However, a few cladistic hypotheses to species level for fish families exist for comparing the geologic and phylogenetic pattern and there have been only a few attempts to make this comparison (e.g., Winterbottom, 1985). Vicariance biogeography of deep sea fishes is almost completely unstudied. Hulley (1998) has made a start with some Antarctic myctophids but no attempts have been made in the tropical Indo-Pacific.

One of the key biogeographic patterns of the tropical Indo-Pacific is the diversity maximum centered around the Indo-Malay-Philippine archipelago. An understanding of this pattern will require a synthesis of at least four explanations for this diversity: 1) The fact that this area has the greatest concentration and area coverage of highly productive, shallow tropical habitats of anywhere on earth (which may dampen the extinction side of the diversity equation). 2) Sea level lowering during the Pleistocene would have resulted in an effective barrier between the Indian and Pacific Oceans (Flemming, 1986) and created a number of isolated basins in Southeast Asia (McManus, 1985), setting the scene for potential allopatric speciation. 3) A diversity of lithospheric plates meet in Southeast Asia. 4) The direction of lithospheric plate movement is mostly toward Southeast Asia rafting taxa into the area like gigantic conveyor belts. The challenge for the future is to construct phylogenetic hypotheses that will allow tests of these factors. In addition, a closer look at areas such as central and northeast Kalimantan where substantial faunal changeover of freshwater fishes occurs is warranted.

The future of systematic studies of Indo-Pacific fishes lies in the ability to uncover novel characters that will be useful in phylogenetic inference at all levels, but particularly for intra-ordinal and intra-familial relationships. Many of the traditional morphological



approaches to inferring phylogeny have suffered from a lack of useful characters. Soft anatomical characters and features in addition to osteology have not yet been adequately studied and may contain much useful phylogenetic information. A closer study of the parasites of fishes may also uncover useful co-evolutionary relationships. Molecular approaches to systematics also show promise but much theoretical work needs to be done to resolve conflicts between morphological and molecular cladograms. Some combination of morphometric, meristic, osteological, soft anatomy, and molecular characters may be necessary to muster an adequate number of characters to produce well corroborated phylogenies. In support of molecular studies, greater development of non-formalin preserved material will become increasingly important.

### CONSERVATION

One of the biggest challenges to systematists is the issue of conservation. Our key role is to describe and study evolutionary relationships of biological diversity. However, the diversity of fishes in the Indo-Pacific is threatened by habitat disruption and overexploitation. Coastal anthropogenic activities have resulted in widespread loss of valuable reef, mangrove, seagrass bed, and estuarine habitats. The life history strategy of certain groups such as elasmobranchs may not be resilient enough to sustain current and future levels of exploitation. Very little is known about the population dynamics and levels of exploitation of elasmobranchs in the Indo-Pacific. In addition, the species limits and alpha taxonomy of many widely exploited batoid fishes in the Indo-Pacific is poorly known (P. Last, pers. comm.).

It would be simplistic to state that it is unnecessary to worry about the conservation of deep sea fishes because if their habitat is threatened, the rest of the aquatic world will be beyond repair. One deep sea habitat that is causing concern is the tops of sea mounts, where repeated trawling may damage low numbers of unique taxa. Serious consideration should be given to protecting representative sea mounts in different areas of the world.

Freshwater fishes are in greater danger of extinction because of proximity to man than most marine fishes. Many human activities directly impact rivers and lakes. Deforestation, dams, canals, mining, introduction of exotic species, industrial effluents, domestic effluents, and other forms of habitat disruption have posed a serious threat to the survival of many freshwater fishes in the Indo-Pacific (Kottelat and Whitten, 1996).

Systematists can help conservation efforts by using their expertise to identify global biodiversity hotspots and integrating their work with international agencies such as the Species Survival Commission of the IUCN. Systematists can provide leadership and through international agencies convey pertinent information to government and non-government agencies that can mobilize conservation efforts. Areas of special endemism and megadiversity areas such as those in Southeast Asia must be clearly delineated to set priorities. Potential habitat problems must be identified in order to develop effective strategies for fish conservation. These strategies should integrate other organisms aside from fishes in order to maximize impact and need for support. The iconic nature of fishes in many cultures needs to be emphasized to elevate fishes as high profile animals in multiple use aquatic reserves.

Systematists must also set research priorities to delineate genetic diversity and the need for its conservation. For example, the study of intraspecific variation may be impor-



tant in understanding the limits of biodiversity. Systematists should obtain the information needed to offer sound advice regarding the impacts on biodiversity and genetic contamination to those seeking to introduce exotic species for aquaculture and other purposes. The assumption that marine reserves can be both self-recruiting and seed centers for unprotected areas needs to be critically tested. The potential use of captive breeding programs and their impact on conservation needs to be critically evaluated.

The collective efforts of systematists should not be underestimated in their ability to impact conservation issues. A good example of this is the influence around 50 visiting scientists had in a policy decision regarding industrial development of an area in the Philippines. During the 1995 workshop in the Philippines in support of production of the FAO Identification Guide to the Western Central Pacific, attending scientists signed a letter recommending that a cement factory not be located in an ecologically sensitive coastal area in Bolinao, Philippines. This letter, and evidence gathered by the Marine Science Institute of the University of the Philippines helped conserve this coastal area in spite of strong economic pressure (E. Gomez, pers. comm.).

## CONCLUSIONS AND RECOMMENDATIONS

It is ironic that in the face of the current global outcry to preserve biodiversity, support for systematic ichthyology is diminishing. Future ichthyological research requires both funding and trained practitioners. Unfortunately, the number of fish taxonomists around the world is decreasing. For example, within Australia the number of full time fish taxonomists has decreased from 14 in 1990 to 9 in 1997, with further decreases likely (Paxton *et al.*, 1997). Most other museums and institutions involved in systematic research of Indo-Pacific fishes are also experiencing dwindling support for taxonomists and their research. The net result is a lack of interested young scientists and a future with fewer systematic ichthyologists than are needed. Another serious problem is the lack of funds to cover publication costs. There is dwindling support for printing of long systematic monographs and the color plates that are needed to accurately report the diversity of life.

In order to meet the present biodiversity challenge in the current environment of decreasing support, fish systematists will need to pursue innovative funding strategies. Institutions and societies may need to band together and form a proactive lobby to better inform the public and policy makers about the role of systematic research in the understanding and conservation of biodiversity. Large exploration and research undertakings may need to rely on international cooperation. Developed countries will need to coordinate with less developed countries in efforts such as museum building. This will serve to harness local research manpower, bring conservation projects close to areas where they are needed most, and utilize international aid programs to further research needs. Ichthyologists will have a stronger justification for research costs if projects are done in cooperation with other biologists. This could be accomplished by coupling collection efforts with other target taxa and projects with other disciplines such as ecology and behavior. Purely academic and simple natural history interests may be less defensible for funding unless the results can be used for public education. In general, systematic ichthyologists must learn to demonstrate that their work is valuable in the challenge to preserve biodiversity and hence, for the public good.

**Acknowledgements.** - We thank all the following who offered valuable comments during the workshop on the "Future of Ichthyological Research in the Tropical Indo-Pacific" at the Fifth Indo-Pacific Fish Conference in Noumea: H. Bleher, F. Bonhomme, A. Brautigam, C. Burridge, K. Clements, T.H. Cribb, T. Donaldson, W. Eschmeyer, R. Fricke, T. Gill, M. Gomon, Shunping He, P. Hulley, P. Last, J. Leis, K. Matsuura, K. Martin-Smith, E. Murdy, P. Musikasinthorn, M. Rigby, C. Roberts, B. Séret, K. Samantaratay, A. Stewart, R.E. Watson, J. Widodo. We are most grateful to T. Clarke of the University of Hawaii, B.B. Collette of the U.S. National Marine Fishery Service Systematic Laboratory, C. Ferraris of California Academy of Sciences, E. Fujii of Tokai Fisheries, B. Hulley of the South African Museum, C. Klepadlo of Scripps Institution of Oceanography, K. Martin-Smith of Aberdeen University, M. McGrouther of the Australian Museum, J.E. Randall of the Bishop Museum, J. Rivaton of ORSTOM Noumea, and T.R. Roberts for providing information useful in preparing this account. Finally, we thank the organizers of the Fifth Indo-Pacific Fish Conference whose hard work made the conference both enjoyable and a great success.

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## Workshop 4

### FISH COLLECTION MANAGEMENT

Chairperson: **Jean-Claude HUREAU**

FROESE R., BAILLY N., CORONADO G., PRUVOST P., REYES Jr. R. & J.-C. 697-705  
HUREAU. - A new procedure to evaluate fish collection databases.

*Une nouvelle procédure d'évaluation des bases de données de collections de poissons.*

HUREAU J.-C. - Co-ordination and standardisation of the management rules for 707-716  
fish collections.

*Coordination et standardisation des règles de gestion pour les collections de poissons.*



## **A NEW PROCEDURE TO EVALUATE FISH COLLECTION DATABASES**

by

Rainer FROESE (1), Nicolas BAILLY (2), Grace CORONADO (1),  
Patrice PRUVOST (2), Rodolfo REYES Jr. (1) & Jean-Claude HUREAU (2)

**ABSTRACT.** - A procedure is presented that compares some of the information typically contained in fish collection databases (scientific name, identifier, locality, coordinates) with reference information compiled in FishBase, a large biological database on finfish available on CD-ROM. The procedure detects possible errors in spelling, locality, and identification. It can be used to assign a reliability indicator to collection records, detailing the confidence in the given scientific name, identifier, area, country, and coordinates. For records with country assignment or with coordinates, various maps can be produced to visually detect possible errors in locality or identification, and to check for marine species being recorded from land, and vice-versa. Preliminary experiences in applying the procedure to subsets of several collection databases are presented.

**RÉSUMÉ.** - Une nouvelle procédure d'évaluation des bases de données de collections de poissons.

Les auteurs ont mis au point une procédure qui permet de comparer certaines informations habituellement présentes dans les bases de données de collections de poissons (nom scientifique, déterminateur, localité, coordonnées géographiques) avec les informations présentes dans FishBase, une grande base de données biologiques sur les poissons, disponible sur CD-ROM. La procédure détecte les possibles erreurs d'orthographe, d'origine et d'identification. Elle peut être utilisée pour attribuer un indice de fiabilité aux enregistrements de collections, en détaillant la confiance que l'on peut avoir dans le nom scientifique, le déterminateur, la zone géographique, le pays et les coordonnées. Pour les enregistrements qui font référence à un pays ou qui possèdent des coordonnées géographiques, des cartes peuvent être produites afin de détecter visuellement des erreurs de localisation ou d'identification, ou pour vérifier que des espèces marines ne sont pas enregistrées en eau douce et vice-versa. Des tests préliminaires d'application de la procédure à des sous-ensembles de plusieurs collections sont présentés.

**Key-words.** - Fish Collections, Databases, Reliability indicator, Errors.

The importance of museum collections for the study of biodiversity is now widely acknowledged. It is estimated that at least 3.8 million lots of fishes exist in North American museums (Poss and Collette, 1995). About 47% of the lots were computerized in North America in 1990, with a strongly increasing trend (Poss and Collette, 1995). In Europe the number of fish specimens held in museums was estimated at 7-8 million individuals in

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The present paper is ICLARM Contribution n°1491.

1990 (Kottelat *et al.*, 1993), which may result in about one million lots if an average number of 8 specimens per lot is assumed. Additional large collections exist in Australia, Japan, South Africa and South America (Hureau, 1996). The total number of lots in the world may reach 10 million. About 60 collection databases were accessible through the Internet in October 1997.

The quality of the available information about the fish samples is highly variable, with some having been used in a recent revision by world experts, and others not having been looked at for more than 200 years. Most museums are understaffed and curators have a hard time just keeping up with the cataloging of recent acquisitions. Systematically re-identifying each specimen other than in the context of revisionary work seems not to be an option.

Thus, we propose here to compare the information available in typical collection databases with corresponding reference tables in FishBase to assign automatically a reliability indicator that flags misspellings and doubtful scientific names, likely misidentifications and range extensions, as well as records where the identification and locality are most likely correct. This information can be used to prioritize curatorial work or to exclude doubtful records from analyses.

## MATERIAL AND METHODS

FishBase is a large biological database with key information on 17,600 species of finfish, as of October 1997 (Froese and Pauly, 1997). FishBase 98 was released after this study and contained 54,000 names for 20,000 species (Froese and Pauly, 1998). For the purpose of this study, FishBase 97 contained an OCCURRENCE table with 53,563 museum collection records from several museums in Europe and USA. It also contained extensive synonymies, distribution records by FAO area and by country, and a preliminary list of fish taxonomists, with indication of their family of expertise. This information was used to derive a combined reliability indicator for the stated scientific Name, Identifier, FAO Area, Country, and Coordinates (NIACC). The NIACC reliability indicator was assigned automatically to existing records, based on criteria explained below. For each category a number between 1 and 5 was assigned, with the following meaning:

### Scientific name

- 1: unambiguous (in the synonymy of one species only);
- 2: unambiguous, but has been misapplied (in more than one synonymy, species may be difficult to identify);
- 3: ambiguous (matches no name in FishBase or points to more than one species, especially if no author was given);
- 4: not available (e.g., for 'sp.');
- 5: not yet evaluated.

### Identifier

- 1: expert of respective family (has published - as first author - a revision of the family or a genus within the family);
- 2: expert (has published a taxonomic revision of another family);
- 3: other identifier (has not published a revision as first author);
- 4: not available (no identifier stated);
- 5: not yet evaluated.



**FAO area**

- 1: species known from stated FAO area;
- 2: (category not used);
- 3: species not known from stated FAO area;
- 4: not available (no FAO area stated);
- 5: not yet evaluated.

**Country**

- 1: species known from stated country;
- 2: species not reported from stated country, but list of countries for this species is incomplete in FishBase;
- 3: species not known from stated country (and list of countries for this species in FishBase is complete);
- 4: not available (no country stated or name does not match an ISO country name);
- 5: not yet evaluated.

**Coordinates**

- 1: coordinates of locality are within (fresh) or adjacent to (marine) the geographic range of the stated country (the range is a rectangle of the nearest latitudinal and longitudinal degrees that include the country; for marine species, the range is extended by 4 degrees in each direction);
- 2: (category not used);
- 3: coordinates are not within or adjacent to the stated country;
- 4: not available (no coordinates or no country stated);
- 5: not yet evaluated.

**Tables**

The following reference tables in FishBase were used:

- The SYNONYMS table with 46,318 names for 17,640 species, classified as: junior synonym, misidentification, misspelling, original combination, new combination, questionable, other.
- The EXPERTS table, which contains the names of currently 387 taxonomists for which the FishBase REFRENS table contains a publication that is classified as revision of a genus or a family, and of which they are the first author. The EXPERTS table also states the respective families of these revisions.
- The FAOAREAS table with 35,678 records of FAO statistical areas from which a species has been reported, classified either as native, endemic, introduced, extirpated, reintroduced, or unclear.
- The COUNTRY table, which states for each of the 17,640 species in FishBase the ISO country names from which they have been reported. Note that currently such country lists are complete for only about 50% of the species in FishBase.
- The COUNTRREF table, which contains for each of the ISO countries, as well as for a number of islands the nearest latitudinal and longitudinal full degrees that include the country.

## RESULTS

### Scientific name

About 83% of the scientific names were unambiguous synonyms (category 1). Eleven percent of the names referred to one valid species, but had also been misapplied to other species, thus having a higher chance for possible misidentifications (category 2). Four percent of the names could not be linked to any synonym in FishBase and thus may either be misspelled or not yet contained in FishBase, or refer to more than one biological species, such as in the case of *Alectis indica*, which is a misspelling of *Alectis indicus* (Rüppell, 1830), and also refers to *Alectis indica* (Cuvier, 1833), which is a junior synonym of *Alectis ciliaris* (Bloch, 1787). Since authority names are often omitted in collection databases, the name could not be assigned to a valid species (category 3). FishBase contains a routine to find misspellings in scientific names (Froese, 1997). Two percent of the names did not provide specific epithets (category 4).

### Identifier

For the purpose of this study we created an EXPERTS database with 387 family experts. We standardized the names in 13,948 of the 16,025 records for which Identifier names were given in the FishBase OCCURRENCE table. Of these records, 11% were identified by family experts, 73% were identified by other experts, and 10% were identified by persons of unknown experience. Note that not all museums have a system in place that keeps track of subsequent identifications.

### FAO area

About 61% of the lots stated FAO areas that were compatible with the established distributional range of the species (category 1). Eight percent gave areas outside the established range (category 3). A closer look at these records revealed three possible reasons: 1) a wrong FAO area had been assigned to the locality stated in the museum database; 2) the FAO area was compatible with the locality stated in the record, and thus was either a range extension or a misidentification; and 3) the area was correctly assigned and compatible with the established range, but the FAOAREAS table in FishBase erroneously did not contain a record. In this case, FishBase was corrected. About 28% of the records had no FAO area assigned to them (category 4), and three percent of the records could not yet be evaluated because the scientific name had no match.

### Country

About 68% of the country names provided matched a country assigned to the species in the FishBase COUNTRY table (category 1). Twelve percent did not match a record in the COUNTRY table, but the list of countries for that particular species was known to be incomplete in FishBase (category 2). Four percent of the countries provided were not contained in lists that were complete for the species, and thus were either range extensions or misidentifications. In a few cases, countries were found to be missing from lists that were supposed to be complete, and this was corrected in FishBase. Fourteen percent of the records had no country name assigned (category 4), and two percent of the records could not yet be evaluated because the scientific name did not match a valid species (category 5).

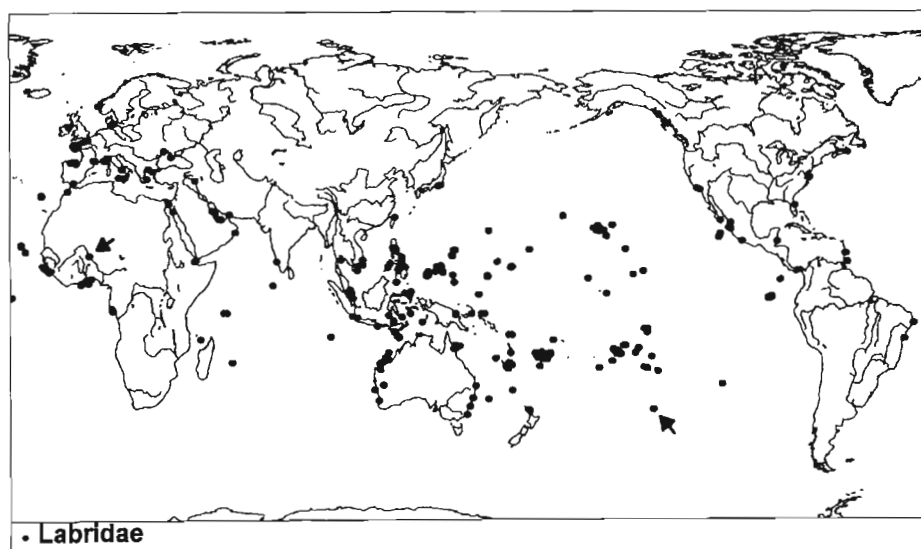


Fig. 1. - Example of a map showing point data for Labridae. Note that some points were erroneously plotted on land whereas other points were plotted in the open ocean where the occurrence of wrasses is highly unlikely.

### Coordinates

Of the records in the data set, 85% had coordinates assigned to them, due to a preference for geo-referenced records in FishBase. About 80% of the records had coordinates that fell inside the range of the specified country (category 1). Three percent of the coordinates were outside the range of the specified country (category 3), indicating an error either in the coordinates or in the stated country. Fifteen percent of the records had no coordinates assigned to them (category 4), and two percent had no country assigned and thus could not be evaluated by this method (category 5).

### NIACC

Five digits with 4 or 5 possible entries each allow for 2,000 possible combinations for the NIACC reliability indicator. Of these, only 301 were assigned by the algorithm in the current study. The most common combination was NIACC 14111, which was assigned to 37% of the collection records, suggesting highly reliable records with so far unevaluated identifier. Similar good marks were NIACC 24111 for 3%, NIACC 12111 for 4%, NIACC 12121 for 2% and NIACC 11111 for 1.4% of the records.

Some combinations gave considerable insight in the evaluation of the collection record. For example, NIACC 11111 refers to a specimen with an unambiguous name, identified by a family expert, and reported from a locality, country and area within its established range. NIACC 11131 indicates a similar well identified specimen from a locality in a new country that is within its broader range, i.e., most likely a new record (Table I). NIACC 23331 indicates a species with a name that has been misapplied before, identified by an unknown identifier, and reported from a locality clearly outside the established range, i.e., most likely a misidentification. We think that these examples demonstrate why a '5 telling digits' indicator is more useful than a summarized single digit indicator.

Table I. - Collection records marked by the algorithm as probable range extensions. The suggested new countries are given in square brackets. The distributional range is stated as contained in FishBase 97. See text for discussion of the table.

<i>Abudefduf bengalensis</i> [Hong Kong]	Indo-West Pacific: Eastern Indian Ocean, north to Japan, south to Australia.
<i>Abudefduf sordidus</i> [Palau]	Indo-Pacific: Red Sea and East Africa Hawaiian and Pitcairn Islands, north to Japan, south to Australia.
<i>Apistogramma cacauioides</i> [Colombia]	South America: Suriname.
<i>Bellator militaris</i> [Cuba]	Western Atlantic: North Carolina to southern Florida and northern Gulf of Mexico in USA; south to Yucatan in Mexico.
<i>Caelorinchus caribbaeus</i> [Trinidad and Tobago]	Western tropical Atlantic from Cape Hatteras to northern Brazil. Absent in Straits of Florida, uncommon to the north and along Antillean chain.
<i>Caelorinchus multispinosus</i> [China]	Southern Japan to East China Sea.
<i>Caelorinchus occa</i> [Antigua Barbados]	Central North Atlantic: From Florida Straits to northeastern South America (uncertain). One record from Bermuda. Atlantic: southern Africa (must be confirmed), from Faroe Channel to Cape Verde Is. (Ref. 3587).
<i>Caelorinchus parallelus</i> [New Caledonia (2 records)]	Indo-west Pacific: southern Japan, East China Sea, and the Philippines, but may extend into Indian Ocean, Australia and New Zealand.
<i>Chronis weberi</i> [China]	Indo-Pacific: Red Sea and South Africa (Ref. 4391) to Line Is. and Samoa, north to southern Japan, south to New Caledonia; Palau in Micronesia.
<i>Chrysiptera glauca</i> [Cook Islands, Palau (2 records)]	Indo-Pacific: East Africa to the Line and Pitcairn Is.; Australia northwards to Japan; throughout Micronesia.
<i>Chrysiptera rex</i> [Hong Kong]	Indo-West Pacific: Eastern Indian Ocean (Scott and Ashmore Reefs), Ryukyu Is., Taiwan, Philippines, Palau, Indonesia, New Guinea, New Britain, Solomon Is., Vanuatu, New Caledonia, and the Great Barrier Reef.
<i>Cirripectes castaneus</i> [Hong Kong, Kiribati]	Indo-West Pacific: Red Sea to Tonga, north to southern Japan; south to Lord Howe Is.; Palau, Ifaluk, and Kapingamarangi in Micronesia.
<i>Cirripectes polyzona</i> [Palau (3 records)]	Indo-Pacific: South Africa to Kiribati, north to Japan; south to Rowley Shoals and the southern Great Barrier Reef, throughout Micronesia.
<i>Cirripectes quagga</i> [Palau (2 records)]	Indo-Pacific: South Africa to Tanzania, east to Henderson Island, Pitcairn and the Hawaiian Is.; north to China; south to the Great Barrier Reef, Australia.
<i>Cirripectes stigmaticus</i> [Kiribati]	Indo-Pacific: From Mozambique to Kenya, throughout the Indian Ocean and western Central Pacific to the Marshall and Samoa Islands.
<i>Dascyllus flavicaudus</i> [Tahiti]	Southeastern Central Pacific: Southeastern Oceania including Society Is., Tuamotu Is., Pitcairn Group, and Rapa.
<i>Nezumia convergens</i> [Ecuador (4 records)]	Eastern Pacific: Ranges from the Gulf of California, Mexico south to Chile, including Cocos and the Galapagos Islands.
<i>Pomacentrus emarginatus</i> [Solomon Is.]	Indo-Australian: Waigiu (off west New Guinea) and Palau.
<i>Stegastes nigricans</i> [Viet Nam]	Indo-Pacific: Red Sea and East Africa to the Tuamotu, Marquesas, and Line Islands, north to the Ryukyu and Bonin Islands, south to New Caledonia and Tonga; throughout Micronesia. Excluding the Hawaiian Islands (Ref. 7247).
<i>Ventrifossa macropogon</i> [Puerto Rico, St Kitts Nev. (2 records)]	Western tropical Atlantic from off Guyana into the Caribbean and the Gulf of Mexico, and in the Atlantic off northeastern Florida. Common to the south of the Gulf of Mexico, but relatively rare in the Gulf and Gulf stream.
<i>Ventrifossa mucocephalus</i> [Haiti]	So far known only from the western Caribbean, the Straits of Florida off Cuba, and the Atlantic off northeastern Florida.
<i>Ventrifossa nigrodorsalis</i> [New Caledonia (5 records), Viet Nam]	Known from southern Japan, Taiwan, Philippines, and parts of Indonesia (Borneo, Halmahera). Slight morphological variation seen in specimens from Japan and Indonesia, but not sufficient to recognize additional taxa.
<i>Ventrifossa petersoni</i> [Myanmar]	Indo-Pacific Ocean.

Several combinations related to the unfinished status of the evaluation process (see below), e.g., NIACC 14411 (11%), indicating that no FAO area were available, or NIACC 14311 (2%), indicating that the name, country and coordinates were correct, but there was a probably erroneous mismatch of country and FAO area in the collection database.

## DISCUSSION

One of the problems with museum collection databases is a lack of consistent standards. For example, identifier names are stated with one, two, or without initials, with leading or trailing initials, with spelled out prenames, with additional information such as years or collaborators, etc. For example, the unique list of 807 different entries in the Identifier field of the OCCURRENCE table contained 8 variations of what should have been 'Randall, J.E.'. Similarly, there are rarely any standards used for geographic areas and country names. In addition, these fields are usually filled manually by typing in the information, instead of selecting it from pick-lists, and consequently there are numerous typos. Standardizing the information in these fields is the first task before the NIACC quality indicator can be assigned. The printout of an alphabetic list of unique entries in a given field is an efficient way to approach this work. We want to stress, however, the importance of preserving the original information (original identification, locality, subsequent identifications, etc.), with the specimen in the jar as well as in the collection database.

There are already some reliability indicators in use in collection databases. A system applied by Australian museums assigns 5 levels from 'Highly reliable identification' to 'Identification superficial', based on the 'taxonomic experience of the identifier, their knowledge of the group considered, and the amount of effort given to make the identification'. The quality level is assigned manually by data encoders or by the identifiers them-

Table II. - Collection records that were identified by the algorithm to be probable misidentifications (NIACC 23331). The countries stated in the collection records are given in square brackets. The descriptive text is the range given in FishBase 97.

<i>Johnius dussumieri</i> [Hong Kong]	Indian Ocean: from the southern coast of South Africa (not the Red Sea) eastward to the Andamans. Other records outside this area are doubtful.
<i>Carcharhinus wheeleri</i> [New Caledonia, Solomon Islands]	Western and Central Indian Ocean: Natal, South Africa to Somalia, the Gulf of Aden, and Red Sea. Termed as <i>C. amblyrhynchus</i> by Wheeler (1962) and <i>C. spallanzani</i> by Bass, D'Aubrey and Kistnasamy (1973). Very close to <i>C. amblyrhynchus</i> and may prove to be not distinct from that species.
<i>Malacocephalus laevis</i> [Hawaii]	Eastern Atlantic: Faroe Is. to South Africa. Reported from Iceland. Western Atlantic: Straits of Florida to Brazil, Gulf of Mexico and Caribbean Sea. Indian Ocean: Arabian Sea, Bay of Bengal, off Maldives and off East African coast. Western Pacific: Indonesian area and Australia. <i>M. hawaiiensis</i> , <i>M. luzonensis</i> , and <i>M. nipponensis</i> are closely related to <i>M. laevis</i> and may eventually prove to represent populations of this species, if a comprehensive comparison of material from the Atlantic, Indian Ocean and Pacific is done.

selves (Williams *et al.*, 1996). The MUSE collection database system which is used by several museums in North America has a Yes/No field for 'questionable' records, which refers to the confidence that the cataloger has in the identification. Also, locality records can be marked as 'proofed' if the cataloger is satisfied with the accuracy and quality of the data (Dave Catania, pers. comm.).

Our criterion for inclusion in the EXPERTS database (first author of a generic or family revision) was a pragmatic one, and we are waiting for feedback to improve on this. One could, for example, argue that also co-authors of such revisions should be considered experts of the respective family. However, we want to stress that the criterion for inclusion should be an objective one, allowing the computer to do the assignment whenever a new revision is entered in the FishBase REFRENS table.

An algorithm has been developed to plot coordinates using the WINMAP software that comes with FishBase (Coronado and Froese, 1997) and write back a file that states for every point whether it fell on 'land' or 'sea'. For example, of 282 occurrence points plotted for Chaetodontidae, 29 fell on land. Some of these were true mistakes, whereas others just reflected the fact that butterfly fishes occur close to the shore and that the often used accuracy of full geographical minutes is not sufficient to avoid such points being plotted on land. Point data by family can also be evaluated on screen, to find obvious outliers (see Fig. 1).

We realize that the reliability of the NIACC indicator itself depends on the quality and completeness of the respective reference tables in FishBase. However, these tables will continuously be improved the more they are used for this and other purposes, and we feel that even in their current status the frequency of errors was relatively low.

Table I lists all records that were classified as likely range extensions or new records for a country (NIACC 11131), with the possible new country indicated in the collection database and the distributional range given in FishBase. Countries in FishBase are assigned to a species if 1) they are mentioned in a distributional range, such as shown in table 1; 2) they are included in a map showing the distribution of a species, or 3) there is a taxonomic reference explicitly naming the countries. The list of countries for a species is marked 'complete' in FishBase if the available sources allow such a statement. As for the records in table I, most countries are within or very close to the given range, making the addition of the new country to the established list highly likely. One record (Tahiti) is an omission in the FishBase country list, because Society Islands are stated in the range. Altogether the algorithm classified these records satisfactorily.

Table II lists all records that were classified as probable misidentifications (NIACC 23331), again with the suggested country and the range given in FishBase 97. As can be seen from the text, these species were already known to be misidentified outside their area, or to be part of a species complex that needs further study and may be one species only. The algorithm thus classified these records correctly.

**Acknowledgements.** - We thank Alice Laborte for implementing the various procedures to assign the NIACC reliability indicator to the existing records. We thank the FishBase team for having assembled the reference tables used in this study. FishBase has been developed with support from the European Commission, DG VIII.

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## CO-ORDINATION AND STANDARDISATION OF THE MANAGEMENT RULES FOR FISH COLLECTIONS

by

Jean-Claude HUREAU (1)

**ABSTRACT.** - For several centuries, the large museums of natural history of the world have had to face a huge increase in their collections in all fields: marine organisms, terrestrial arthropods and vertebrates, plants, geological and palaeontological samples or ethnographic objects, ... Fishes are not an exception to this general rule, because of the frequent collecting expeditions and oceanographic cruises, resulting from scientific programmes. Curators of fish collections have to cooperate and co-ordinate their efforts in order to standardise their management rules. With the development of computerisation of the data linked to the collections, an absolute precision in the collection of data must be approached. Even if non-written rules or tacit agreements already exist for fish collection management, it seems necessary to clarify some aspects of the relationships between museums. The purpose of the present paper is to propose some simple rules that every curator should follow. A debate should take place within the present workshop in order to come to some general agreement.

**RÉSUMÉ.** - Coordination et standardisation des règles de gestion pour les collections de poissons.

Depuis plusieurs siècles, les grands Musées d'histoire naturelle du monde ont dû faire face à un fantastique accroissement de leurs collections dans tous les domaines de l'histoire naturelle: organismes marins, arthropodes et vertébrés terrestres, végétaux, échantillons géologiques et paléontologiques ou objets ethnographiques, ... Les poissons ne font pas exception à la règle générale, en raison des nombreuses expéditions de collecte et des campagnes océanographiques liées aux programmes de recherche. Les conservateurs des collections de poissons se doivent de coopérer et de coordonner leurs efforts afin de standardiser leurs règles de gestion des collections. Avec le développement de l'informatisation des données attachées aux collections, une précision absolue dans la collecte des données doit être un objectif prioritaire. Même si des règles non-écrites ou des accords tacites existent pour la gestion des collections de poissons, il semble nécessaire de normaliser certains aspects des relations entre les musées. L'objectif du présent papier est de proposer quelques règles simples que chaque conservateur pourrait suivre. Ce workshop sur la gestion des collections peut être l'occasion d'un débat fructueux afin d'obtenir un accord général.

**Key-words.** - Fish collections, Management rules, Natural History Museums, Computerisation.

For more than ten years, the study of biodiversity has been one of the questions of the day and all scientists, particularly the taxonomists, are requested to build data sets on zoological and botanical species. In fact, the oldest long-term biological data sets are the collections (and their associated data) preserved in the museums. These collections have been made over a considerable number of years, sometimes several centuries, like in the Museum national d'histoire naturelle (Paris), The Natural History Museum (London) or the

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Zoological Institute (St Petersburg), and they were primarily established as taxonomic references in natural history. However, with the development of biodiversity studies and the improvement of molecular techniques, the collections represent a long-term data base and their role is now overstepping the bounds of systematics. The above mentioned museums, together with the National Museum of Natural History, Smithsonian Institution (Washington), constitute the biggest in the world (70 million specimens in Paris and 67 million in London, according to Hureau, 1996), but, taking into account the number of primary or secondary type-specimens, the collections in London and Paris are the richest in the world. However, no less significant collections of specimens, and associated data, are stored in smaller museums around the world, many of which are attached to marine stations, university departments or private institutions.

### WHAT IS A NATURAL HISTORY MUSEUM ?

Before proposing some co-ordination and standardisation of the management rules for fish (and other ?) collections it seems necessary to briefly define the role of a natural history museum. Such a museum has three fundamental responsibilities: heritage conservation, research, and public education.

#### **Heritage conservation**

For several centuries (since the beginning or mid-18th century for the oldest museums), the large natural history repositories in the world preserved specimens collected during many cruises, expeditions and surveys, the cost of which being immeasurable. The effort of thousands of scientists and technicians, developed during several hundreds of years by several tens of countries, is also impossible to evaluate. Many species preserved in the museums are now extinct in nature or, at least, in their original habitat. So the responsibility of museums in conserving the natural heritage is especially fundamental as repositories of past biodiversity and as a testimony of the human and financial efforts of past generations.

#### **Research**

Systematics is the main research field of the museums. Collections are continuously used (they are "alive"), increased in size, complemented in order to offer a permanent scientific tool for the study of species evolution and identification for ecological investigations. With the development of new techniques (e.g., molecular systematics, biochemistry, and ... still unknown techniques), their role is constantly increasing. During the 19th century, it was considered that museums should shelter a maximum number of species, each one being represented by one or few specimens. At present it is clear that large series of specimens must be studied for each species.

In the field of biodiversity and evolution, four levels of biodiversity studies have been undertaken (Chauvet and Olivier, 1993): (1) intra-specific diversity, with measurements of genetic diversity for the distinction between variants of the same species; (2) specific and inter-specific diversity between species within a given geographical area based on inventories of living species; (3) ecological or ecosystem diversity; and (4) evolution of diversity over geological time scales.

In other words, it is possible to say that fish research in museums should identify those taxa on which we have sufficient information now to guide decisions on the conser-

vation of water bodies to protect biodiversity. We should also focus research on testing critical hypotheses that will help to understand further the measures necessary to conserve the aquatic natural environment of the world.

### **Education of the public at large**

Scientists working in museums should be aware that one of their main roles is the dissemination of knowledge and the education of the public through various media, like scientific publications, books for a larger audience and permanent or temporary exhibitions. Another means of dissemination of knowledge consists in the free availability of data attached to the collections (metadata) and in the collection exchanges and loans for scientific and public exhibition use. But scientists must be aware that scientific collections and those for exhibition are not the same: only the former ones will be considered in this paper.

## **WHAT IS A FISH COLLECTION ?**

Ideally, a fish collection is an assemblage of specimens systematically organised into species lots. It is not just an inventory and a load of stored specimens, but it forms a reference pool usable by scientists throughout the world. Such a collection may be world-wide in its scope or focused into smaller units of country or geographic area. For some years, collections of fishes (like collections in all other fields of natural history) have grown up due to the activities of oceanographic cruises and international expeditions, which have strained the storage capacity and budget of museums (Hureau, 1993). At the same time, the development of new techniques, such as molecular systematics, has affected the care of collections, usually with undesirable high cost consequences. It is paradoxical that, just as there is a new interest in the data accumulated within collections, and in new technologies for using and disseminating these data, many organisations, for which curation is not one of their primary duties, are wishing to dispose of valuable material that museums have been also holding for many decades with many financial and human difficulties. Even the specialised museums, however, are now having to consider the financial implications in accepting new material. Compared to the budget of large national or international scientific programmes (like space programmes or oceanographic programmes), maintaining and developing natural history collections require almost nothing.

## **MANAGEMENT OF COLLECTIONS**

Museums possess collections of great richness. A variety of more than 70 million objects are preserved in the Paris Museum (Hureau *et al.*, 1995) and 67 million are stored in London (Bodmer *et al.*, 1993). Speaking only about fish, 118 collections of fish were studied by Collette and Lachner (1976) and by Poss and Collette (1995) in United States and Canada: they determined the total number of specimens housed in these collections to be 64 million. In Europe, the total number of specimens has been estimated to be 7 to 8 million specimens in 125 institutions (Kottelat *et al.*, 1993). If we compare the number of nominal species represented by these collections, about 20,250 are present in the United States and Canada, and 25,250 in Europe (Hureau, 1996). About 19,000 **primary** types of

fishes are preserved in the seven larger North American museums (LACM-Los Angeles, USNM-Washington, UMMZ-Michigan, AMNH-New-York, CAS-San Francisco, NMC-Ottawa, ROM-Toronto) and about 25,000 in the 10 larger European museums (BMNH-London, MNHN-Paris, ZIL-St Petersburg, ZMB-Berlin, ZMH-Hamburg, RMNH-Leiden, NMW-Wien, NRM-Stockholm, ZMUC-Copenhagen, ISNB-Bruxelles): a little less than 11,000 in Paris, more than 8,000 in London.

All these collections were normally initially catalogued in different ways, but generally on a card-index file and/or a register in each laboratory or department or division involved in storing collections. To date, no general protocols for registering exist, so each institution, or even each laboratory within the same institution, manages its collections independently. Thus with no agreed national protocols, it is not surprising that international protocols do not exist. This lack of international agreements is one of the factors inhibiting the wider availability of information on where specimens are to be found and which data exist concerning these collections. The introduction of computerisation to collection management will force all curators and keepers of collections to adopt agreed protocols in presenting the data. Some efforts have already been made especially for the 60 fish collection databases accessible through the Internet in October 1997 (Froese *et al.*, present workshop, p. 698): e.g., in France, we are assembling in only one database, all the information available on the types of fishes present in all French museums. This database, GICIM (Computerised Management of the Ichthyological Collections of the Museums), was planned as early as 1977 (Daget, 1977; Daget and Hureau, 1979, 1980, 1981) but was actually born in 1983 (Hureau and Murail, 1988): it has been accessible through the Internet since 1994. But we must admit, to incorporate the types that are not in Paris, the task was not too difficult: apart from the Museum national d'histoire naturelle in Paris, with its nearly 19,000 primary and secondary types of fishes, the other museums that have fish types, i.e., La Rochelle, Lyon, Monaco and Strasbourg, house only 230 types. But we thought useful that the international community of ichthyologists could have access to the data concerning all these types located in France.

The most important agreement necessary concerns the information minimum made available to the ichthyologists. In the databases, whatever the format or the software used, this minimum must be given for each specimen or lot of fishes. A list of these metadata has been proposed earlier (Hureau, 1993; Hureau *et al.*, 1995); they can be presented under several headings (Fig. 1):

#### **Taxonomic data**

Necessary data include the present taxonomic position of a specimen: i.e., family-, genus- and species-names, author's name(s), date of publication of the valid species name. For the types, it is also necessary to indicate the type-status and the original taxonomic position published by the author of the nominal species. Most ichthyologists search for a type under its original published name, not under its present name. Consequently a database should preserve the history of the denominations given to a specimen, since it has been registered in the collections.

#### **Specimen and collection data**

These data give information on the specimen itself and may help to know if a loan is possible: museum number (which means that all the specimens must be registered!), locality and geographical origin as precisely as possible (e.g., geographical coordinates, hydrographical basin and/or river name, marine area), station number or field

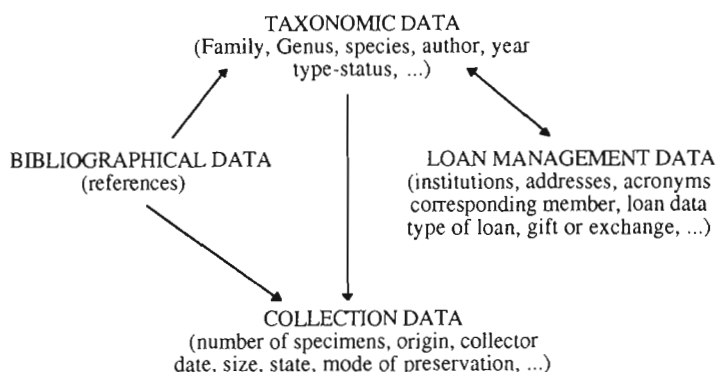


Fig. 1. - Type of data which should be stored in a database of a fish collection, and relationships between these data.

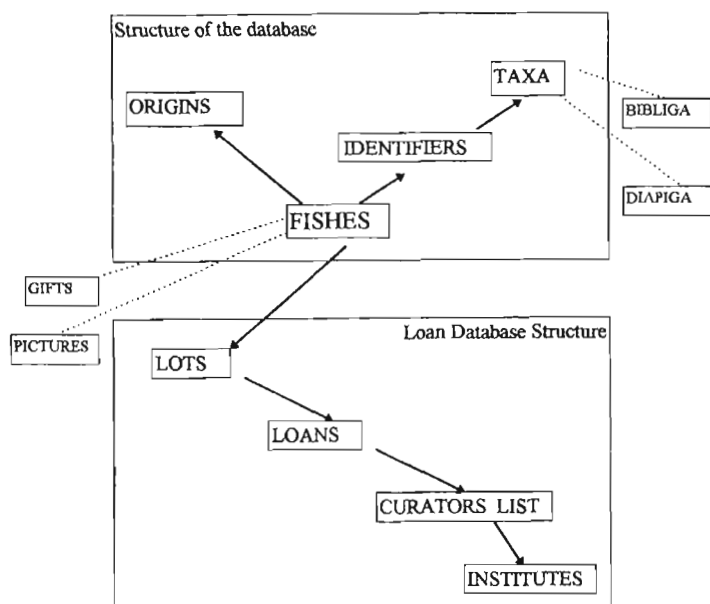


Fig. 2. - GICIM database structure with links between the various tables (existing links: →; future links: .....). BIBLIGA and DIAPIGA are respectively two existing bibliographical references and slides databases. Tables ORIGINS, TAXA, IDENTIFIERS, FISHES, LOANS are used daily for requests with the ACCESS Computer Software. GICIM is a relational multitable database.

number, depth of capture (for marine species), date of collection (day, month, year), name of collector, name of identifier and/or reviser, size (total and/or standard length; if a specimen is several meters long, it cannot be sent by mail!), mode of preservation (alcohol, other preservative, stuffed, herbarium of fish, ...; fragile specimens cannot be mailed), state of the specimen (in order to know if it can be requested on loan), number of specimens in the lot (if only one or few specimens exist in the collection, it is unnecessary to request a loan for clearing and staining or for anatomical dissection).

### **Other data (of minor importance)**

Some information may be useful but not absolutely necessary, such as presence of a photograph and/or X-ray in the archives (it is unnecessary to do it again if it already exists; an X-ray can be requested on loan like the specimen itself), sex and sexual stage, stomach content, vernacular name, collecting gear or sampler, nature of the habitat, nature of the bottom, ...

### **Loan management data**

In order to manage carefully loans to the other ichthyologists, some information is necessary to ensure the security of the loan and of the specimen, to engage the responsibility of both parties (curator and scientist requesting a loan). These data are: the name of the institution in which the requesting scientist is working (with address and international acronym), the corresponding member (name, first name, precise address) to whom the loan should be sent (probably, it is in that field that an agreement must be made with an official form and a published list of the curators responsible of a collection), the loan number, the name of the curator of the collection from which the loan is sent, the duration of the loan (open long-term exchange or gift) or, if it is a short-term loan for examination, with or without some restrictions (e.g., authorisation or not to dissect, to clear and stain the specimen, to take a sample for DNA or other analysis).

### **Bibliographical data**

References to the published papers or books in which the specimen is mentioned is also useful to the scientists who consult a collection database. These references generally give useful knowledge on the conditions in which the specimen was collected. Some of these papers are also good revisions of a taxon, at the family or genus level.

All the above mentioned data are represented in figure 1 and detailed information as they are present in the GICIM database are indicated in figure 2, which gives an overall scheme of the structure of that database.

## **FUNDING, UTILISATION AND STORAGE**

The amount of money required to fund a major museum is significant. In a previous paper (Hureau, 1993), I tried to evaluate the negative and positive factors that affected the care and preservation of the Paris Museum collections. It is unlikely that it is significantly different in other countries. One of the negative factors is the competition between "whole-organism science" and new technologies, or to be more precise, the opposition between organismic biology and molecular biology. But this opposition should be considered now as a recent past difficulty, because systematics is increasingly richer because of the additional information generated by disciplines such as biochemistry, molecular biology, cytogenetics, enzymology, physiology and so on. Museums have now a more and more important role to play and the collections should be largely open to utilisation and consultation by scientists from many new disciplines, other than morphology and anatomy. The move towards applied and/or directed science will probably not reverse in the immediate future and it is important for the scientific users of museums' facilities, i.e., the international research community, to ensure that the right priorities are set for utilisation of increasingly limited resources. This means that museums should open their doors largely to scientists, and simultaneously that international protocols should be estab-

lished in order to avoid the rapid deterioration of collections patiently created and increased during decades or centuries.

Storage of collections becomes increasingly problematical, not only because of the increase in the volume of collections but also because more frequently society invokes new rules on, for example, health, fire and safety which, although designed for industry, have a major impact on museums' funding. For example, the Zoological gallery in Paris, which housed the spirit collection until 1985, represented a significant fire and explosion risk. The building that housed this collection for almost one hundred years could not pass the latest regulations. It was therefore necessary to build a new structure matching to new safety standards. This needs also to be discussed by the curators who should be aware of that problem.

### SOME QUESTIONS RAISED BY COLLECTION COMPUTERISATION

More and more fish curators are computerising their collections and it is a very good initiative, even a necessity, with growing collections. But the greatest problem facing the world's museums in computerisation of their records is the cost, not of equipment, but of the staff-time to enter data and, most importantly, to check them for accuracy. R. Froese *et al.*, in the present volume (pp. 697-705), have given a paper on this important question of accuracy of data. Some protocols have already been applied by ICLARM (International Center for Living Aquatic Resources Management) in Manila, for FISHBASE and some others are still in discussion.

In databases, there is a problem which is not completely solved. It concerns the geographical names: which names should be used? English names? French names? Spanish names? local names? This problem has to be discussed because it is not so simple as it might appear at first glance. The same problem also exists for the vernacular names that replace far too often the scientific names in many publications, mostly from our American colleagues. Which names must be used in the databases? Firstly, the Latin or scientific name, but if such databases are to be made available to the public, it is clear that vernacular names must be used also. Most of the databases are now planned to be used world-wide, so which vernacular names should be recorded? Concerning the commercial species, I think it is necessary to use FAO names, even if they are not always well chosen, but these names are already widely employed for commercial use, for statistics and fisheries.

The production of a database on the collections' metadata is a huge enterprise. At a time when funds are not unlimited, the efforts of all the scientists and taxonomists should be joined. In my opinion, it is more useful, not only for the scientific community but also for a larger public, to collaborate in few (if not one) databases usable all over the world. The scientists should not hesitate to give their data freely, about collections which are not their own property, to one central institution able to produce a large database, as complete as possible. It seems ridiculous to me when I discover projects based on small, restricted and unknown databases. In our discussion, I wish to raise again this problem, which is probably one of the most crucial, if we want to save some funds.

## CONCLUSIONS

Collection-based research is one of the oldest forms of biological science and the Museum collections stored and curated around the world have the potential to be an unevaluated source of long-term historical data (Dauvin and Lamshead, 1997). Traditionally, these collections and associated data, were designed for taxonomic research, but the challenge is now to make them equally useful and accessible to answer ecological, biogeographical and other biodiversity questions that need an historical perspective. The cost of maintaining collections continues to mount and both specialised and non-specialised museums have difficulties in balancing their budgets. New technologies (e.g., chemical analytical techniques, cytogenetics, enzymatic polymorphism, ...) allow the recovery of molecular data from stored specimens, have greatly confirmed the value of traditional museum collections (Hureau, 1993), and will make collections a treasure-trove of historical data. The computerisation of museums' collection records is critical and developments in computers softwares allow the management of important databases. Incorporation of new data acquisition and analysis are also particularly important to define homologous descriptions. This should permit comparison and computerisation of descriptions usable in expert systems, image analysis and video systems. But problems remain with the cost of:

- entering data into computers, a cost that must be balanced against other scientific priorities;
- data validation, which can take a great deal of time but which is absolutely essential;
- curation of fish collections for which the budgets remain clearly insufficient, especially in term of staff-time; and
- staff, particularly if we consider the dramatic reduction of specialists for many taxonomic groups. This is the reason why it is so important to incorporate their knowledge into expert systems before it is lost.

Discussions raised at the end of the present workshop will address these various aspects of fish collections management which may be summarised as follows:

**Collection registration.** - Mode of numbering, paper or card file, register book, computer file, database, ... what is the best? Everybody agrees to consider that databases constitute the basic tool of any curator and collection keeper.

**Information data** (or metadata). - Which ones are the basic data common to all collections? Such a basic data list can easily be elaborated.

**Collection use.** - By whom, for what kind of research? Many workshops, symposia or world congresses may be dedicated to that subject. It is clear that collection uses have evolved since the 18th Century. The entire world community (scientists, environmentalists, economists, the larger public, ...) now is aware of the necessity of preserving biodiversity and, as a consequence, to maintain collections of natural history and their precious associated metadata.

**Buildings' safety.** - How the curators may be involved? Even if curators are directly concerned by this question, the problem is mostly for the people in charge of science management to deal with.

**Data accuracy.** - How to estimate the accuracy of the data in a fish collection: identification should be confirmed periodically by specialists and the metadata accuracy is of the responsibility of the curators.



**Geographical and vernacular names.** - Which language ? It seems necessary now to use in the databases several names (one basic name in English (?) and other names in several languages). All these names being synonyms for a geographical locality or a taxon, this procedure could avoid any difficulty when preparing a request to a database.

**Budget for fish curation.** - How to solve that problem ? The question remains open, but it is clear that international co-operation is a good means to avoid duplications and useless expenses.

**Staff and specialists scientists.** - How to replace them ? or can we replace them, at least partially ? Curation of collections will always necessitate staff time, but a better choice in the methods of curation could avoid some expenses. Specialists are retiring, and their numbers are continuously decreasing. Our governments and people in charge of science must be aware of that problem. Even if it becomes necessary to develop expert systems to avoid any information loss, specialists in taxonomy will remain essential.

**Co-ordination within the numerous databases.** - How can we merge the efforts of ichthyologists of the world ? Already now, several large databases on fishes are in development and use. A better information transfer among specialists and non-specialists should be developed. Co-ordination between existing databases could partially avoid some expenses. Some efforts at different levels (European, American, international) are already in progress, but this question will be difficult to solve if each curator and scientist is not convinced of the necessity to collaborate widely and clearly with his colleagues from any country.

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## Workshop 5

### FISH POISONING: WHAT DO WE KNOW ?

Chairperson: **Jordi MOLGÓ**

- MOLGÓ J. - Introduction to the workshop. 719-720  
*Introduction à l'atelier.*
- MOLGÓ J., BENOIT E., LEGRAND A.-M. & A.S. KREGER. - Bioactive agents involved in fish poisoning: An overview. 721-738  
*Agents bioactifs responsables des empoisonnements dus aux poissons: une vue d'ensemble.*
- LEWIS R.J., JONES A. & J.-P. VERNOUX. - HPLC/Mass spectrometry and HPLC/MS/MS methods for the detection of multiple ciguatoxins. 739-744  
*Méthodes d'HPLC couplées à la spectrométrie de masse pour la détection de diverses ciguatoxines.*
- BENOIT E., MATTEI C., LEGRAND A.-M. & J. MOLGÓ. - Ionic basis of the neurocellular actions of Pacific ciguatoxins implicated in ciguatera fish poisoning. 745-758  
*Bases ioniques des actions neurocellulaires des ciguatoxines du Pacifique.*
- PAULLAC S., SASAKI M., NAAR J., INOUE M., BRANAA P., CRUCHET P., CHINAIN M. & A.-M. LEGRAND. - Immunochemical methods for ciguatoxins detection in Pacific herbivorous and carnivorous fish. 759-773  
*Méthodes immunochimiques de détection des ciguatoxines dans les poissons herbivores et carnivores du Pacifique.*

- POLI M.A., MUSSER S.M. & S. HALL. - Laboratory diagnostics of brevetoxin and ciguatoxin intoxications in humans: Case reports and sampling considerations. 775-781

*Analyses de laboratoire pour diagnostiquer les intoxications humaines par les brévétoxines et ciguatoxines: exposés de cas et conditions des prélèvements.*

- ANGIBAUD G. - Clinical recognition of ciguatera fish poisoning in New Caledonia. 783-786

*Signes cliniques de la ciguatera en Nouvelle-Calédonie.*

## **INTRODUCTION TO THE WORKSHOP 5. FISH POISONING: WHAT DO WE KNOW?**

by

Jordi MOLGÓ (1)

The workshop was held during the 5th Indo-Pacific Fish Conference in Noumea and was dedicated to Professor **Takeshi Yasumoto** who, by virtue of his many years of scientific inquiry of the highest quality, has made major contributions to our knowledge of the chemistry and biogenesis of marine toxins.

During this workshop the bioactive agents and toxins involved in fish poisoning incidents in the Indo-Pacific and Caribbean region were reviewed by the chairman, with emphasis on their origin, chemical structure and mechanisms of action. Furthermore, the detection, clinical recognition and public health problems arising from consumption of contaminated fish were discussed.



Some of the workshop speakers, in ORSTOM gardens (from left to right): Takeshi Yasumoto (Japan), Mark Poli (USA), Jordi Molgó (France), Evelyn Benoit (France), Dominique Laurent (New Caledonia) and Richard Lewis (Australia). Serge Pauillac (French Polynesia) and Gilles Angibaud (New Caledonia) were not available for the photo.

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The biogenetic origin and the chemical structure of ciguatoxins, including the numerous analogs characterized by high performance liquid chromatography (HPLC) and mass spectrometry (MS), was highlighted by **Takeshi Yasumoto (Sendai, Japan)**. He also addressed clupeotoxism, a highly fatal and mysterious intoxication resulting from ingestion of sardines or herrings, and the recent isolation of carchatoxins A and B implicated in shark poisoning.

**Richard J. Lewis (Queensland, Australia)** presented evidence, using a turbo-assisted, gradient reverse phase HPLC/MS/MS method, that Pacific and Caribbean ciguatoxins can be detected at levels as low as 0.05 ppb in crude fish extracts, using as little as 2.5 g of flesh. The method can be used to confirm the presence of ciguatoxin in fish suspected of causing ciguatera and will be invaluable in the establishment of much needed rapid screening methods for ciguatera.

The ionic basis of ciguatoxins' action on nerve membranes was documented by **Evelyne Benoit (Gif-sur-Yvette, France)**. She discussed the selective action of these toxins on voltage-dependent sodium channels, which leads to an increase in  $\text{Na}^+$  permeability and to an alteration of the osmotic equilibrium between the intracellular and the extracellular media. In addition, she emphasized the mode of action of hyperosmotic D-mannitol on nervous tissue, an agent that is now widely used in the treatment of ciguatera fish poisoning.

The strategy used to develop a laboratory, antibody-based assay that could be scaled up to perform routine, cost-effective mass screenings of Pacific herbivorous and carnivorous fish prior to consumption was addressed by **Serge Pauillac (Tahiti, French Polynesia)**, who also discussed current hapten immunochemical knowledge and guidelines for future application to ciguatoxins-specific antibody-based assays.

**Mark A. Poli (Frederick, U.S.A.)** provided firm evidence that a family of Caribbean ciguatoxins was responsible for an outbreak of fish poisoning affecting soldiers serving with the Multinational Force in Haiti.

**Gilles Angibaud (Noumea, New Caledonia)** closed the workshop with a retrospective analysis emphasizing the importance of clinical diagnosis in the management of ciguatera fish poisoning.

## BIOACTIVE AGENTS INVOLVED IN FISH POISONING: AN OVERVIEW

by

Jordi MOLGÓ (1), Evelyne BENOIT (1),  
Anne-Marie LEGRAND (2) & Arnold S. KREGER (3)

**ABSTRACT.** - Consumption of a variety of fish is associated with an increasing number of human intoxications and diseases which are important public health problems worldwide. In this brief review we analyze the main types of fish-poisoning, with emphasis on the modes of action of the bioactive agents involved in those incidents. Ciguateras are a family of polyether toxins responsible for ciguatera, a complex form of fish poisoning characterized mainly by gastrointestinal, neurological and cardiovascular disorders. At present, ciguatera constitutes one of the largest seafood toxicities associated with consumption of many species of tropical and subtropical fishes widespread in the Indo-Pacific Oceans and Caribbean sea. Progress has been made in the determination of the chemical structure and the mode of action of ciguateras. These toxins cause persistent activation of voltage-dependent  $\text{Na}^+$  channels, increase neuronal excitability and neurotransmitter release, impair synaptic vesicle recycling, and modify  $\text{Na}^+$ -dependent mechanisms in many cell types. Scombrotoxic fish poisoning is a totally preventable disease that results mainly from consumption of Scombridae fish containing unusually high levels of histamine due to poor attention to principles of hygiene during distribution and storage of fish. Tetrodotoxin is one of the most lethal seafood toxins known. This toxin is present in most puffer fish species (family Tetraodontidae) living in the Indo-Pacific area. Even though puffer fish are easily recognized, there are still many outbreaks of tetrodotoxin poisoning yearly. Tetrodotoxin also has been identified in freshwater puffer fish (*Tetraodon palembangensis* and *T. fangi*) living in tropical inland waters. Recent findings indicate that the toxic principle of *T. fangi* is not tetrodotoxin, but saxitoxin. Both toxins are known to block, in a highly specific manner, voltage-dependent  $\text{Na}^+$  channels in excitable membranes. Blockade of  $\text{Na}^+$  channels prevents action potentials from propagating along axons, nerve terminals and muscle fibers, which leads to inhibition of nerve impulse-evoked neurotransmitter release in chemical synapses. Other forms of fish poisoning (e.g., those resulting from the presence of palytoxin in fish or those implicating new marine toxins) are also reviewed and discussed.

**RÉSUMÉ.** - Agents bioactifs responsables des empoisonnements dus aux poissons: une vue d'ensemble.

La consommation d'une grande variété de poissons est associée à un nombre croissant d'intoxications et de maladies humaines qui sont des problèmes importants de santé publique largement répandus dans le monde. Dans cette revue, nous analysons les principaux types d'empoisonnement par les poissons en insistant sur les modes d'action des agents bioactifs impliqués dans ces incidents. Les ciguateras sont une famille de toxines polyéthers responsables de la ciguatera, une forme complexe d'empoisonnement par des poissons caractérisée principalement par des troubles gastro-intestinaux, neurologiques et cardio-vasculaires. Actuellement, la ciguatera constitue l'une des plus grandes toxicités de l'alimentation marine répandues dans les Océans de l'Indo-Pacifique et dans la Mer des Caraïbes

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qui soient associées à la consommation de nombreuses espèces de poissons tropicaux et subtropicaux. Des progrès ont été faits dans la détermination de la structure chimique et du mode d'action des ciguatoxines. Ces toxines provoquent une activation persistante des canaux  $\text{Na}^+$  dépendants du potentiel, augmentent l'excitabilité neuronale et la libération de neurotransmetteurs, altèrent le recyclage des vésicules synaptiques et modifient les mécanismes dépendants du  $\text{Na}^+$  dans de nombreux types cellulaires. L'empoisonnement par les Scombridae est une maladie totalement évitable qui résulte principalement de la consommation de viande contenant des taux anormalement élevés d'histamine dus à un manque d'hygiène durant la distribution et le stockage des poissons. La tétródotoxine est l'une des toxines les plus mortelles qui soient connues de l'alimentation marine. Cette toxine est présente dans la plupart des espèces de poissons ballons (Tetraodontidae) vivant dans les régions Indo-Pacifiques. Le fait que les poissons ballons soient aisément reconnaissables n'empêche pas l'apparition chaque année de nombreux cas d'empoisonnement par la tétródotoxine. Cette toxine a été également identifiée dans des poissons ballons d'eau douce (*Tetraodon palembangensis* et *T. fangi*) vivant dans les eaux tropicales intérieures. Des résultats récents indiquent que le principe toxique de *T. fangi* n'est pas la tétródotoxine mais la saxitoxine. Ces deux toxines sont connues pour bloquer, d'une manière hautement spécifique, les canaux  $\text{Na}^+$  dépendants du potentiel dans les membranes excitables. Le blocage des canaux  $\text{Na}^+$  empêche la propagation des potentiels d'action le long des axones, des terminaisons nerveuses et des fibres musculaires, ce qui conduit à une inhibition de la libération de neurotransmetteurs provoquée par l'impulsion nerveuse au niveau des synapses chimiques. D'autres formes d'empoisonnements par des poissons (e.g., ceux qui résultent de la présence de palytoxine dans les poissons ou ceux qui impliquent de nouvelles toxines marines) sont aussi passés en revue et discutés.

Key-words. - Fish poisoning, Shark poisoning, Food poisoning/etiology; Marine toxins, Ciguatera, Ciguatoxins, Tetrodotoxin, Saxitoxin, Palytoxin, Clupeotoxism, Histamine.

Humans are exposed to naturally-occurring toxins by the consumption of a variety of contaminated seafood products, including fish. In some parts of the world, at certain times of the year and under certain conditions, poisonous fish constitute a public health problem. Approximately 400 to 500 species of marine fish may be poisonous to humans after ingestion. Most of these species, but by no means all, are found in coral reefs. Usually their distribution is spotty, in a particular part of the ocean or around a given island. Most poisonous fish are nonmigratory reef fish and can be either herbivores or carnivores. Some of the fish species have tissues which are toxic at all times, others are poisonous during certain periods of the year or in certain geographical areas, while still others have only specific organs which are toxic, and their toxicity may vary with time, location and habitat.

This brief report is intended to review the main types of foodborne diseases caused by poisonous fish, with emphasis on the cellular mode of action of the marine toxins or bioactive agents they contain. It is not possible within the confines of this review to consider in detail the properties of all of the bioactive agents and toxins involved in fish poisoning, but we apologize for any errors of omission.

Fish poisoning may be classified according to: (i) the type of fish or family of fish involved in the poisoning (e.g., puffer fish poisoning), (ii) the clinical signs and symptoms presented by the patients, (iii) the biological structures or target(s) affected, and (iv) the toxin or bioactive agent implicated. During the last few years there has been a breakthrough in our knowledge of the chemical and pharmacological properties of the complex toxins involved. Therefore, we have classified fish poisoning according to the bioactive agent or toxin responsible for the poisoning.



Each of the main clinical syndromes involved in fish poisoning is caused by different types of bioactive agents or toxins produced by miscellaneous species of marine dinoflagellates and bacteria. The increase in the international transport of fish and in international travel by fish consumers has resulted in virtually no human population being free from the risk of fish poisoning. Although records are often incomplete because reports to specialized centers for disease control are scarce, available evidence indicates that ciguatera is responsible for most worldwide fishfood intoxications.

### CIGUATOXIN FISH POISONING (CIGUATERA)

Ciguatera, a form of fish poisoning with a pantropical distribution, has been recognized as the most common foodborne illness associated with the consumption of many species of tropical and subtropical fish contaminated with the ciguatoxin class of polyether toxins. The disease is endemic and of significant public health concern throughout the Caribbean and Indo-Pacific regions, where it has been known for centuries. Although mortality is low ( $< 0.1\%$ ), morbidity is high and symptoms may be debilitating and prolonged. The increase in travel to and from the tropics and in the importation of tropical foodfish has resulted in ciguatera appearing in temperate countries of North America and Europe with increased frequency. The reader is referred to other publications (Yasumoto *et al.*, 1984; Russel and Egen, 1991; Swift and Swift, 1993; Lewis and Holmes, 1993; Tosteson, 1995) for more comprehensive reviews.

Usually, the organisms containing ciguatoxins are located in discrete regions of a reef, and flare-ups of ciguatera are both temporally and spatially unpredictable (Bagnis, 1981; Lewis and Holmes, 1993). The existence of ciguatoxic fish, which are physically indistinguishable from nontoxic fish, has serious implications for public health and for resource development and tourism in many islands.

Events leading to a ciguatera outbreak are initiated by environmental and genetic factors that favor the proliferation of the marine dinoflagellate *Gambierdiscus toxicus* and its production of gambiertoxins. The precise factors involved in the toxicity of the dinoflagellate are yet to be determined. Gambiertoxins and/or ciguatoxins are transferred from the benthos to herbivorous fish and then to carnivorous fish via the marine food chain. The toxins are ingested by and accumulate in fish which, when consumed by humans, ultimately cause ciguatera. The factors that influence the concentration of ciguatoxins that accumulate in fish include the (i) rate of dietary intake, (ii) efficiency of assimilation, (iii) degree and nature of toxin biotransformation and rate of depuration, and (iv) rate of growth of fish (Lewis and Holmes, 1993).

During the last decade, progress has been made in the isolation, purification and determination of the chemical structure of the ciguatoxins present in wild and cultured dinoflagellates (*G. toxicus*), as well as in poisonous fish caught in the Pacific Ocean (Legrand *et al.*, 1989; 1992; Holmes *et al.*, 1991; Murata *et al.*, 1989; 1990; Lewis *et al.*, 1991; 1994; Lewis and Holmes, 1993; Yasumoto *et al.*, 1993; Pauillac *et al.*, 1995; Satake *et al.*, 1996; Yasumoto and Satake, 1996; Lewis and Jones, 1997) and, more recently in the Caribbean Sea (Poli *et al.*, 1997; Vernoux and Lewis, 1997). The data obtained from the above studies indicate that Pacific and Caribbean ciguatoxins extracted from ciguateric fish are composed of two distinct families of heat stable, cyclic polyether toxins having molecular masses of 1,030-1,160 Da, and may account for the clinical syndrome of ciguatera in the Caribbean Sea and Pacific Ocean. Figure 1 shows the chemical structure of ciguatoxin-1B purified from poisonous Pacific fish.

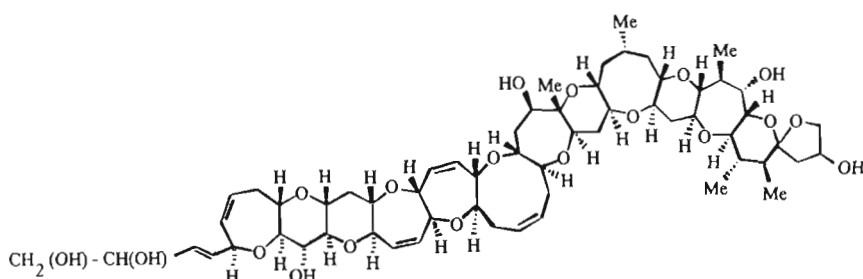


Fig. 1. - Chemical structure of ciguatoxin-1B, which is considered to be the major ichthyotoxin involved in ciguatoxic fish poisoning in the Pacific Ocean.

A model outlining the biotransformation and acid-catalysed spiroisomerisation of the toxins produced by *G. toxicus* has been proposed based on the production of gambier-toxin/ciguatoxin-4A and -4B (Lewis and Holmes, 1993). At the same time, the structure of CTX-3C, a ciguatoxin congener with a new skeletal backbone was isolated from a cultured *G. toxicus* strain (Satake *et al.*, 1993). Later on, ciguatoxin-4A was also isolated from this strain as well as from the flesh of the parrotfish *Scarus gibbus* (Satake *et al.*, 1996), a fish species previously known to contain a typical ciguatoxin congener, named scaritoxin (Chungue *et al.*, 1977). Caribbean strains of the benthic dinoflagellate *G. toxicus* are suspected to be the source of Caribbean ciguatoxins. The extent to which some ciguatoxins are biotransformed as they pass through the marine food chain remains to be determined.

Pharmacological studies have revealed that ciguatoxins target voltage-dependent  $\text{Na}^+$  channels with high affinity. These channels are critical elements for the generation and propagation of electrical signals in most excitable cells, and are also present in non-excitable cells (e.g., glial cells). Voltage-activated  $\text{Na}^+$  channels are plasma membrane proteins that mediate the rapid increase in membrane  $\text{Na}^+$  conductance responsible for the depolarizing phase of action potentials in many excitable cells. As a consequence of

Table I. - Mode of action of ciguatoxins.

Activate voltage-dependent $\text{Na}^+$ channels of excitable membranes (neuronal, skeletal muscle and cardiac muscle) and of nonexcitable cells (Schwann cells and glial cells)
Activate $\text{Na}^+/\text{Ca}^{2+}$ exchange in the reversed mode
Cause spontaneous and tetanic muscle contractions
Increase membrane excitability
Induce membrane depolarization and spontaneous and repetitive action potentials in excitable cells
Induce repetitive, synchronous neurotransmitter release
Produce a transient increase in the quantal content of synaptic responses
Decrease the quantal content and block synaptic transmission
Enhance spontaneous quantal neurotransmitter release
Impair synaptic vesicle recycling
Exhaust neurotransmitter release
Cause swelling of axons, nerve terminals and perisynaptic Schwann cells

ciguatoxin's interaction with Na<sup>+</sup> channels, there is a negative shift in the voltage-dependence of their opening behavior (the so called "activation"). This allows the opening of the ion-conducting pore of the channels at potentials at which it normally remains closed, thus permitting Na<sup>+</sup> movement along the electrochemical gradient. In addition, such channels do not close or inactivate as normal channels do. This persistent ciguatoxin-induced activation of Na<sup>+</sup> channels at the resting membrane potential is responsible for numerous Na<sup>+</sup>-dependent effects (reviewed by Molgó *et al.*, 1992; 1998; see Benoit *et al.* for more details, this volume and references therein). Table I summarizes present knowledge on the mode of action of ciguatoxins.

Ciguatera symptoms depend on ciguatoxin(s) levels, the type of fish (herbivore, carnivore) or sub-product of fish eaten (e.g., soup), the area of the world in which the fish were caught (Caribbean, South Pacific, etc.) and the individual human susceptibility to ciguatoxins.

Epidemiologic characterization of ciguatera has been limited by the lack of a laboratory test to confirm the presence of ciguatoxins. The pathophysiologic features and symptoms of the disease in different areas of the world have been reviewed (Bagnis *et al.*, 1979; Gillespie *et al.*, 1986; Swift and Swift, 1993; Lange, 1994; Glaziou and Legrand, 1994; Quod and Turquet, 1996) and compared with those of other seafood-related syndromes (Levine, 1995). The clinical picture is characterized by a variety of gastrointestinal, neurological and, to a lesser extent, cardiovascular symptoms (Table II).

Neurological symptoms tend to be the most distinctive and enduring, and include sensory changes such as generalized pruritus, circumoral numbness, reversal of hot and cold sensation, and long-lasting weakness and fatigue. Patients with bradycardia and/or hypotension may require urgent care because cardiovascular symptoms may indicate a poor prognosis. Though the symptoms are relatively well-documented, the disease often goes unreported and misdiagnosed. Early symptom recognition has improved the identification and clinical management of ciguatera in endemic and nonendemic areas. However, there is still a need for better diagnostic, preventive, and reporting protocols to more accurately study and understand this diverse clinical syndrome. Though difficult to implement, preventive strategies remain the best defense against ciguatera. Prevention of intoxication, at present, depends upon the avoidance of potential vectors. Immunoassays as well as methods based on mass spectrometry are being developed for detecting even negligible amounts of ciguatoxins in suspect fish flesh (see Pauillac *et al.* and Lewis *et al.*, this volume).

Table II. - Clinical symptoms of ciguatera fish poisoning.

Gastrointestinal	Neurological	Cardiovascular
nausea	paresthesias (extremities and circumoral)	bradycardia
diarrhea	dysesthesia (temperature reversal)	hypotension
vomiting	ataxia	tachycardia
	arthralgia	
	myalgia	
	headache, vertigo and dizziness	
	tremors	
	neck stiffness	
	salivation	
	perspiration	

The treatment of ciguatera is primarily supportive. Intravenous D-mannitol has evolved as a unique remedy for patients with acute poisoning (see review by Palafox, 1992). The management of chronic symptoms continues to be problematic because the pathophysiological basis of symptoms that persist for weeks or years remains to be elucidated.

## HISTAMINE FISH POISONING (SCOMBROID FISH POISONING)

Scombroid fish poisoning is a foodborne intoxication and a form of ichthyosarcotoxism caused by the consumption of large deep-water fish of the family Scombridae (e.g., tuna, *Thunnus thynnus*; mackerel, *Scomber scombrus*; etc.) that are contaminated with unusually high levels of histamine and some biogenic diamines (Morrow *et al.*, 1991). Non-scombroid fish, like mahi-mahi (*Coryphaena hippurus*) in Hawaii, bluefish (*Pomatomus saltator*), sardines (*Sardinops sirm*) (Taylor *et al.*, 1989) and western Australian salmon (*Arripis truttaceus*) (Smart, 1992) also have been reported to produce histamine fish poisoning. Histamine fish poisoning has gradually become a worldwide medical problem and is, after ciguatera, the most common cause of fish poisoning. Noncommercial, recreationally caught fish have been frequently involved in scombroid fish poisoning, and outbreaks due to improper handling and storage of privately caught fish occur yearly (Gellert *et al.*, 1992).

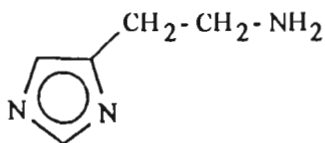


Fig. 2. - Chemical structure of histamine.

Histamine (Fig. 2) formation in fish is due to the decarboxylase activity of some microorganisms, mainly enterobacteria. The main microorganisms involved include *Proteus*, *Escherichia*, *Shigella* and *Salmonella* sp. Bacterial histamine production; e.g., in tuna flesh, can reach levels as high as 60 mg per hour per 100 g of fish tissue at room temperature.

Bacteria involved in the production of histamine can be part of its normal flora or represent a secondary contamination, which may find a favorable environment for growth, if fish are not stored or processed under proper conditions. It is worth noting that fish containing high levels of histamine may not outwardly appear to be spoiled. Histamine levels of 50 mg/100 g of fish flesh are generally considered to be hazardous. Other substances, including putrefactive amines such as putrescine and cadaverine, may also be present in spoiled fish and may potentiate the toxicity of histamine. Interestingly, these potentiators appear to inhibit intestinal histamine-metabolizing enzymes in humans (Taylor, 1986).

During severe outbreaks of scombroid fish poisoning, histamine and N-methylhistamine levels in humans can reach 10 to 40-fold and 15 to 20-fold more than the normal mean values, respectively. Such levels usually decrease within 24 h and are normal about 10 days later.

Pharmacological studies indicate that histamine has a number of actions that result from activation of histamine- $H_1$ ,  $-H_2$  and  $-H_3$  receptors in various organs and tissues. Histamine  $H_1$  receptors are found in smooth muscle and endothelial cells, and in the adre-

nal medulla, heart, and central nervous system. Histamine  $H_2$  receptors are located in gastric parietal cells, vascular smooth muscle and neutrophils, and in the heart and central nervous system; histamine  $H_3$  receptors are present in peripheral nerves of the heart, lung, gastrointestinal tract, endothelium and enterochromaffin cells. Activation of the various types of receptors gives rise to a number of responses (Table III).

The onset of symptoms in scombroid fish poisoning is abrupt, usually occurring within half an hour of ingestion of the affected fish, and is commonly associated with a prominent flush resembling sunburn. The clinical syndrome closely resembles an acute allergic reaction. This similarity in symptoms may result in the diagnosis of scombroid poisoning being missed by clinicians. Patients with the symptom complex may be incorrectly informed that they are allergic to the fish species. In various outbreaks, the implicated fish have been reported to have a peppery taste.

The diagnosis of scombroid poisoning is confirmed by the presence of the clinical syndrome, and by demonstration of high histamine levels in the cooked fish. Scombroid fish poisoning could be misdiagnosed as food allergy or bacterial food poisoning if physicians are not aware of the possibility of histamine poisoning. The nonspecific but characteristic symptomatology of histamine poisoning (Table IV), and previous consumption of fish, should alert physicians to the possibility of scombroid fish poisoning. In some very severe cases, a loss of vision and atrial tachycardia with heart block have been reported (McInerney *et al.*, 1996).

Unless complicated by shock or respiratory distress, supportive treatment with antihistamines usually concludes with a good prognosis (Maire *et al.*, 1992). However, major toxicity may require the same aggressive management as acute anaphylaxis. Histamine analysis of the fish flesh remains the most important step in confirming the diagnosis (Wu *et al.*, 1997).

### TETRODOTOXIN FISH POISONING (PUFFER FISH POISONING)

The toxicity due to consumption of certain puffer fish (Tetraodontidae), ocean sunfish (Molidae) and porcupine fish (Diodontidae) has long been known and is attributed

Table III. - Effects of activation of histamine- $H_1$ , - $H_2$  and - $H_3$  receptors by histamine. \*: Nitric oxide.

Histamine- $H_1$ -receptor	Histamine- $H_2$ receptor	Histamine- $H_3$ -receptor
Smooth muscle contraction	Stimulation of gastric acid secretion	Inhibition of neurotransmitter release
Stimulation of NO* formation	Positive chronotropic and inotropic effects on cardiac muscle	Inhibition of gastric acid secretion
Endothelial cell contraction	Stimulation of adenylyl cyclase	
Increased vascular permeability		
Negative inotropism		

Table IV. - Main symptoms in histamine fish poisoning.

facial or general flushing	diarrhea	oral burning sensation
headache	sweating	itching
nausea	abdominal pain	hives
vomiting	gastrointestinal hyperactivity	hypotension

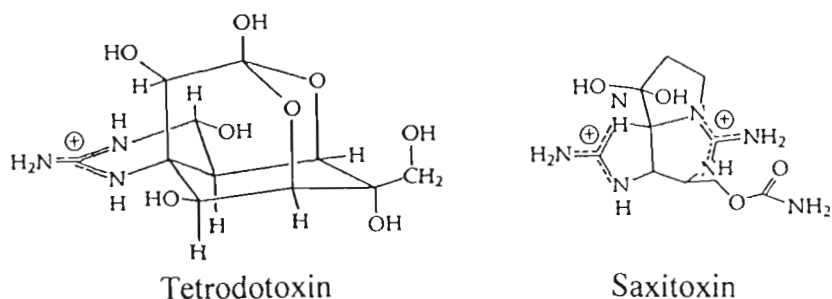


Fig. 3. - Chemical structure of tetrodotoxin and saxitoxin.

to tetrodotoxin. The chemical structure of tetrodotoxin (Fig. 3) extracted from Tetraodontidae, was elucidated independently in Japan and the U.S.A.

Tetrodotoxin is one of the most lethal seafood toxins known. It is present in most puffer fish species and puffer-like fish, which appear to be the only fish universally regarded as poisonous. More than 50 species in the family Tetraodontidae have been reported to cause poisonings in humans, or are known to be toxic under certain conditions (reviewed by Kao, 1966). Tetrodotoxin is concentrated in the ovaries and liver, lesser amounts are found in the intestines and skin, and very small amounts are contained in muscles. In all of the tetrodotoxin-containing species examined, the concentration of tetrodotoxin in the ovaries (which varies between 0.4-1,000  $\mu\text{g/g}$  of fresh tissue) has been higher than in the corresponding male tissues. The amount of toxin in female specimens seems to be related to the reproductive cycle and appears to be greatest just prior to spawning. The observation of a substantial fluctuation in toxin concentration among individuals of the same species living in different habitats led researchers to suspect an exogenous origin for tetrodotoxin. This view was latter supported by reports showing that tetrodotoxin was not found in some puffer fish bred in captivity or caught in the wild (Furhman, 1986; Yasumoto *et al.*, 1986a).

The origin of tetrodotoxin is currently believed to be a broad spectrum of marine symbiotic bacteria (Yasumoto *et al.*, 1986a). Many species in the family Vibrionaceae produce tetrodotoxin, anhydrotetrodotoxin, or both, as revealed by high performance liquid chromatography and mass spectrometry analysis (Simidu *et al.*, 1987). Four strains of tetrodotoxin-producing bacteria isolated from a red alga and from puffer fish have been characterized. Two of these strains are *Listonella pelagia* (*Vibrio pelagius*) biovar II, based on phenotypic characteristics. The other two strains are in the genera *Alteromonas* (*A. tetraodonis*) and *Shewanella* (*S. alga*) (Simidu *et al.*, 1990). Tetrodotoxin-producing bacteria have also been detected in freshwater environments (Do *et al.*, 1993). *Vibrio alginolyticus* also has been reported to produce tetrodotoxin, but the extracted toxin did not react with a monoclonal antibody against tetrodotoxin. Chromatographic analyses have detected high tetrodotoxin concentrations in polypeptone and yeast extracts used in culture media, which has led to question about the bacterial origin of tetrodotoxin (Matsumura, 1995).

In Japan, the puffer fish is considered a delicacy and is prepared by chefs in specially licensed restaurants. Few fatal cases have been reported, during the last 40 years, of puffer fish poisoning from fish served in licensed restaurants in Japan. However, in 1996, three cases of tetrodotoxin poisoning occurred among California chefs who shared con-

taminated fugu (Tetraodontidae) brought from Japan, by a co-worker, as a prepackaged, ready-to-eat product (Anon., 1996a, 1996b). Even though puffer fish are relatively easily recognized, there are still many outbreaks of tetrodotoxin poisoning every year in the Indo-Pacific region, as reported by Poison Control Centers. Most incidents occur during the spawning season of puffer fish; e.g., March to May in southern Asia. An outbreak of tetrodotoxin poisoning after consumption of porcupine fish (*Diodon hystrix*) has also been recently reported (Trevet *et al.*, 1997).

Tetrodotoxin has also been detected in freshwater puffer fish (*Tetraodon leiurus*) (Kodama and Ogata, 1984) living in tropical inland waters (Halstead, 1967). Puffer fish poisoning after consumption of the freshwater puffers *T. fangi* and *T. palembangensis* has also been documented in Thailand (Laobhripart *et al.*, 1990). Considerable toxicity was detected, by bioassay in the skin, eggs, muscle, liver and intestines of *T. fangi*, and tetrodotoxin was believed to be the cause of the poisoning (Saitanu *et al.*, 1991). However, a recent report indicated that the toxic principle of *T. fangi* is saxitoxin rather than tetrodotoxin (Sato *et al.*, 1997). Saxitoxin has been detected in the liver of the puffer fish *Taki-fugu pardalis* (Kodama *et al.*, 1983). This toxin and its derivatives are well known as dinoflagellate toxins (Schantz, 1986) involved in the highly fatal poisoning called paralytic shellfish poisoning.

Pharmacological studies have revealed that the water-soluble heterocyclic guanidines tetrodotoxin and saxitoxin, even though chemically distinct (see Fig. 3), have very similar biologic activities and act as specific blockers of Na<sup>+</sup> channels in excitable membranes. Both toxins inhibit Na<sup>+</sup> channel ion transport by binding reversibly to a common receptor site that is thought to be located near the extracellular opening of the ion conducting pore of the Na<sup>+</sup> channel (Schantz, 1986; Kao, 1986; Narahashi *et al.*, 1994). The main characteristics of the mode of action of both toxins are depicted in table V.

The diagnosis of tetrodotoxin poisoning is usually documented by the identification of puffer fish and/or by the detection of tetrodotoxin in the ingested fish. The clinical signs and symptoms vary widely and begin shortly after the ingestion of fish containing the toxin (Lange, 1990; Lau *et al.*, 1995; Yang *et al.*, 1996). The onset of symptoms

Table V. - Mode of action of tetrodotoxin and saxitoxin.

Block Na <sup>+</sup> channels in excitable membranes, without any effect on other types of voltage-activated and transmitter-activated ion channels
Prevent action potential from propagating along myelinated and unmyelinated axons and muscles
Block nerve impulse-evoked neurotransmitter release
Bind to a common site in the Na <sup>+</sup> channel protein
Na <sup>+</sup> current block results from individual Na <sup>+</sup> channel block in an "all or none" manner

Nausea, emesis, facial paresthesias, numbness
Areflexia (nonreactive pupils)
Reduced sensory and motor nerve conduction velocities
Generalized weakness
Progressive paralysis of the whole body
Respiratory failure

Table VI. - Clinical symptoms of tetrodotoxin fish poisoning.

depends on the concentration of tetrodotoxin ingested, and paresthesias and generalized muscle weakness are common complaints (Table VI).

Variations in the clinical features can be accounted for by large differences in the amount of toxin present in fish. Usually, when severe symptoms appear within a few minutes after fish ingestion, death may occur within 20-30 min in the absence of medical support. When symptoms appear after 24 h, there is a fairly good chance of recovery. Proper therapy is mandatory to successfully treat cases of tetrodotoxin poisoning. Treatment is based on symptoms, and life support (e.g., mechanical ventilation for several days) may be required. Improvement of the patient's clinical condition depends essentially on the reversibility of tetrodotoxin binding to voltage-gated Na<sup>+</sup> channels. Without adequate therapy, patients may have serious morbidity or even succumb. In some outbreaks the mortality rate has approached 60 percent; however, the average fatality rate usually is about 20 percent.

Outbreaks of tetrodotoxin poisoning following the consumption of marine organisms other than puffer fish have been rare. However, several outbreaks of tetrodotoxin poisoning following the ingestion of gastropod molluscs have been recently reported (Yang *et al.*, 1995; Kanchanapongkul and Krittayapoositpot, 1995). Furthermore, because tetrodotoxin, like saxitoxin, blocks voltage-dependent Na<sup>+</sup> channels in biological membranes, it is currently impossible to differentiate between these two toxins solely on the basis of the patients' symptoms. Since the clinical symptoms of puffer fish poisoning and paralytic shellfish poisoning cannot be distinguished clinically (i.e., they constitute an entity), the name pelagic paralysis has been proposed to designate such a clinical condition (Mills and Passmore, 1988; Kanchanapongkul and Tantraphon, 1993).

### PALYTOXIN IN FISH POISONING

Palytoxin is a marine toxin produced by zoanthid coelenterates of the genera *Palythoa* and *Zoanthus*, which are widespread throughout the Caribbean, Pacific and Western Atlantic Oceans (Moore and Sheuer, 1971; Gleibs *et al.*, 1995). Palytoxin also has been found in a sea anemone (Mahnir and Kozlovskaja, 1992) and certain crabs (Yasumoto *et al.*, 1986b; Gleibs *et al.*, 1995) and fish. The entry of the toxin into the human food chain through the consumption of poisonous fish results in human disease.

Palytoxin is a complex, polyhydroxy long-chain macromolecule (Fig. 4) and is one of the most potent marine compounds toxic for mammals.

Table VII. - Clinical symptoms of palytoxin fish poisoning.

Spinal seizure-like syndrome with tonic contractions of all muscle groups
Muscle spasms associated with a remarkable elevation of serum enzymes associated with tissue damage; e.g., creatinine phosphokinase, lactic acid dehydrogenase and glutamic oxaloacetic transaminase
Convulsions
Extreme pain
Myoglobinuria
Respiratory distress, dyspnea
Respiratory failure
Death (2-4 days after fish consumption)



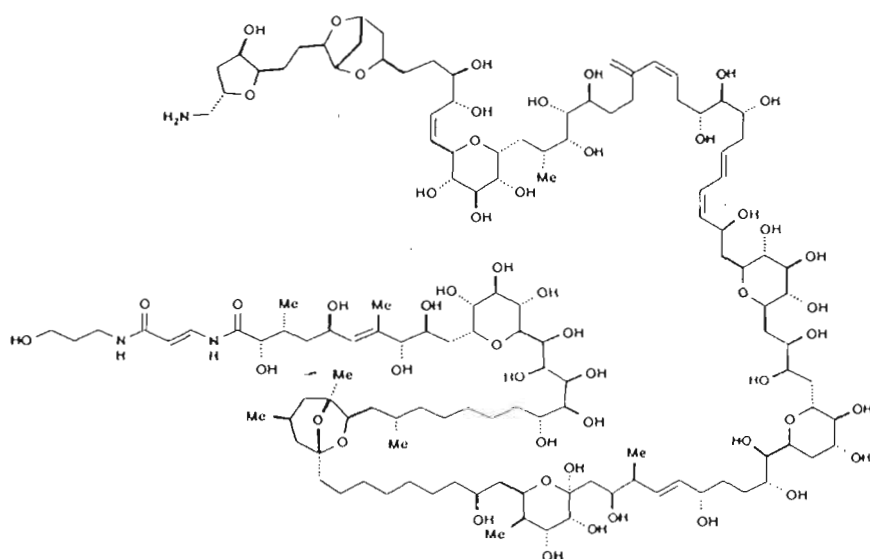


Fig. 4. - Chemical structure of palytoxin.

Trigger fish (Balistidae) are common inhabitants of tropical shores in many areas of the world. The high risk and potential danger of consuming such fish has been repeatedly reported, and their sale has been prohibited in French Polynesia and Hawaii (Tinker, 1978; Bagnis *et al.*, 1982). Human poisoning caused by trigger fish has long been regarded as a form of ciguatera (Halstead, 1967). However, the acuteness and severity of the poisoning, as described by Bagnis (1970), strongly suggests the involvement of a toxin other than ciguatoxin. Table VII summarizes the main signs and symptoms of poisoning with palytoxin.

The occurrence of palytoxin in the viscera of trigger fish (*Melichthys vidua*) collected at Pingelap Atoll (in Ponape in the Federated States of Micronesia) has been demonstrated using high performance liquid chromatography and thin layer chromatography (Fukui *et al.*, 1987). Furthermore, homogeneous palytoxin purified from trigger fish was indistinguishable from reference palytoxin isolated from the marine zoanthid *Palythoa tuberculosa*. Palytoxin has also been shown to be present in the viscera of a file fish (*Alutera scripta*) of the family Monacanthidae, and the toxin source was identified to be ingested *Palythoa* (Hashimoto *et al.*, 1969).

Table VIII. - Mode of action of palytoxin. \*: Single channel conductance expressed in pico Siemens.

Induces activation of small (9-25 pS *), non-selective cationic channels, which triggers secondary activation of $\text{Ca}^{2+}$ channels and $\text{Na}^+-\text{Ca}^{2+}$ exchange
Induces neurotransmitter release from nerve terminals, irrespective of the neurotransmitter involved
Causes contractions of skeletal and smooth muscle cells
Raises intracellular $\text{Ca}^{2+}$
Palytoxin-induced channels are blocked by ouabain, which inhibits (Na,K) ATPase
Has hemolytic activity

Palytoxin has also been implicated in human poisoning after consumption of smoked mackerel (*Decapterus macrosoma*) of the family Scombridae (Kodama *et al.*, 1989). The presence of palytoxin was detected in the fish extracts by chromatographic analysis by the method of Yasumoto *et al.* (1986b) and confirmed by radioimmunoassay using the method of Levine *et al.* (1988). A similar food poisoning incident due to the consumption of parrotfish (*Ypsiscarus ovifrons*) containing palytoxin has been reported (Noguchi *et al.*, 1987).

Palytoxin produces a broad range of pharmacological effects *in vivo* and *in vitro* (Wu and Narahashi, 1988; Habermann, 1989; Shimahara and Molgó, 1990; Rouzaire-Dubois and Dubois, 1990; Sauviat, 1992; Frelin and Van Renterghem, 1995; Kim *et al.*, 1995). Table VIII summarizes the main characteristics of the mode of action of palytoxin.

### CLUPEOTOXISM

Clupeotoxism is a form of fish poisoning characterized by a high mortality rate, caused by consumption of sardines and herrings belonging to the family Clupeidae and anchovies belonging to the family Engraulidae, which has been differentiated from ciguatera on the basis of clinical symptoms. The onset of symptoms (Table IX) usually is very rapid and death may occur within a few hours. During the last 30 years, several outbreaks have been reported in tropical insular areas of the Pacific and the Caribbean. Due to its sporadic and erratic occurrence, the causative toxin(s) has been difficult to identify; however, based on the species involved and the feeding behaviour of the fish, it has been suggested that the toxin(s) may originate in plankton. Recent studies indicate that clupeotoxism is caused by palytoxin or its analogs (Yasumoto *et al.*, 1997). Most probably, the benthic dinoflagellate *Ostreopsis siamensis* is the progenitor of the toxins (Usami *et al.*, 1995; Yasumoto *et al.*, 1997). *Ostreopsis* spp. are distributed worldwide (Faust *et al.*, 1996) as components of tropical and subtropical marine coral reef-lagoon environments, and they are thought to be potential progenitors of toxins responsible for fish poisoning (Nakajima *et al.*, 1981; Tosteson *et al.*, 1986; Holmes *et al.*, 1988; Quod, 1994). Further studies are needed to verify the involvement of *Ostreopsis* spp. in palytoxin production and fish poisoning.

Table IX. - Clupeotoxism symptoms.

sharp metallic or bitter taste	cold, clammy skin	violent headache
nausea	hypotension	hypersalivation
vomiting	cyanosis	muscular cramps
abdominal pain	vertigo	dyspnea
severe diarrhea	tingling of the tongue and lips	progressive muscular paralysis
feeble pulse	numbness	convulsions
tachycardia	nervousness	coma and death
chills	dilated pupils	

## NEW AGENTS IMPLICATED IN FISH POISONING (SHARK POISONING)

The flesh of some sharks like the Greenland shark (*Somniosus microcephalus*) is known to be toxic to humans and animals, especially when in fresh condition. Analysis of toxic fractions obtained from the flesh has revealed the presence of large amounts of trimethylamine oxide but no other substances that could be directly responsible for the poisoning (Anthoni *et al.*, 1991). The clinical symptoms appear to be due to acute trimethylamine poisoning arising from intestinal reduction of trimethylamine oxide.

The first severe outbreak of shark poisoning (98 deaths and a case-fatality ratio of 20%) was reported after the ingestion of a single shark in November 1993, in Manakara, a middle-sized town on the southeast coast of Madagascar (Boisier *et al.*, 1994; Habermehl *et al.*, 1994). The shark involved in this poisoning was identified as *Carcharhinus leucas*. There were no previous reports of similar poisonings in the area, and fishermen in Manakara usually ate this kind of shark without illness. Furthermore, the shark and its meat were not found to possess any unusual characteristics (e.g., contamination with chemicals or bacteria).

The first clinical signs in the Madagascar outbreak appeared within five to ten hours after ingestion, and the patients presented neurological symptoms almost exclusively, the most prominent being a constant, severe ataxia. Gastrointestinal problems, like diarrhea and vomiting were rare (Ramialiharisoa *et al.*, 1996).

Two lipid-soluble toxins were isolated from the shark's liver and they were tentatively named carchatoxin-A and -B (Boisier *et al.*, 1995). The chromatographic properties of the toxins were distinct from ciguatoxins, and their structural profiles are now under investigation (Yasumoto *et al.*, 1997).

## CONCLUSIONS

We conclude that the consumption of a variety of fish is associated with an increasing number of human intoxications in many areas of the world. These diseases result from the actions of specific toxins which have accumulated in fish, but which are produced by dinoflagellates and/or bacteria, and are transmitted through the marine food chain to humans. Although important progress has been made in the determination of the chemical structures of the main toxins involved in fish poisoning and in the characterization of their cellular modes of action, we still do not understand all of the multiple factors that cause dinoflagellates to become toxic under certain conditions and in particular habitats.

At the present time, the diagnosis of various types of fish poisoning depends mainly on the clinical recognition of specific signs and symptoms, and when possible, on the identification of the bioactive agents present in the remains of the fish implicated in the poisoning. However, distinguishing fish poisoning from other forms of seafood poisoning may sometimes be difficult when it is based solely on clinical data.

For most of the types of fish poisoning herein described treatment remains non-specific, symptomatic, and supportive because the pathophysiological basis of the toxins actions still are elusive. Further advancement in the understanding of the pharmacology of the toxins is needed in order to improve specific treatments. Finally, the best strategy for preventing fish poisoning would be to implement specific tests for detecting the presence of toxin(s) in hazardous species of fish prior to their consumption.

**Acknowledgements.** - Marine toxin research in the senior author's laboratory was supported by grants from Direction des Systèmes de Forces et de la Prospective and from International Cooperation Program of the European Community.

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## HPLC/MASS SPECTROMETRY AND HPLC/MS/MS METHODS FOR THE DETECTION OF MULTIPLE CIGUATOXINS

by

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**ABSTRACT.** - High performance liquid chromatography (HPLC) / mass spectrometry (MS) was used to identify ciguatoxins and ciguatoxin congeners present in the viscera of ciguateric moray eels (*Gymnothorax javanicus*) collected in the central Pacific Ocean (P-CTX) and in the flesh and viscera of ciguateric horse eye jack (*Caranx latus*) of the Caribbean Sea (C-CTX). Multiple ciguatoxin congeners were identified from each source. To improve the sensitivity of detection, HPLC coupled to tandem ion-spray MS (MS/MS) methods were developed that detected sub-ppb levels of C-CTX-1 and P-CTX-1. Partially purified fish extracts spiked with pure P-CTX-1 and C-CTX-1 were subjected to reverse phase HPLC and the ciguatoxins present quantified by MS/MS. HPLC/MS/MS proved a sensitive method, not previously available, for the specific analysis of CTX and related toxins at levels in fish below those that cause human poisoning. These MS-based detection methods will facilitate (I) the development of rapid detection methods designed to screen potentially toxic fish before sale and (II) the study of the biotransformation of CTXs that pass through the marine food chain.

**RÉSUMÉ.** - Méthodes d'HPLC couplées à la spectrométrie de masse pour la détection de diverses ciguatoxines.

La chromatographie liquide à haute pression (HPLC) couplée à la spectrométrie de masse (MS) a été utilisée pour identifier les ciguatoxines et leurs congénères présents dans les viscères de murènes ciguatières (*Gymnothorax javanicus*) collectées dans la région centrale de l'Océan Pacifique (P-CTX), et dans la chair et les viscères de carangues (*Caranx latus*) ciguatières de la Mer des Caraïbes (C-CTX). De multiples congénères ciguatières ont été identifiés à partir de chaque source. Afin d'améliorer la sensibilité de la détection, des méthodes d'HPLC couplée à la spectrométrie de masse en tandem (MS/MS) ont été développées détectant des taux sub-ppb de C-CTX-1 et de P-CTX-1. Des extraits de poisson partiellement purifiés, donnant des pics avec la P-CTX-1 et la C-CTX-1 purifiées, ont été soumis à l'HPLC en phase inverse et les ciguatoxines présentes ont été quantifiées par MS/MS. L'HPLC/MS/MS se montre être une méthode sensible, non disponible antérieurement, pour l'analyse spécifique des ciguatoxines et des toxines apparentées à des taux dans les poissons inférieurs à ceux qui provoquent un empoisonnement humain. Ces méthodes de détection basées sur la MS faciliteront (I) le développement de méthodes de détection rapide destinées à dépister les poissons potentiellement toxiques avant la vente et (II) l'étude de la biotransformation des CTXs qui se produit le long de la chaîne alimentaire marine.

**Key-words.** - Muraenidae, *Gymnothorax javanicus*, Carangidae, *Caranx latus*, Ciguatera fish poisoning, Caribbean ciguatoxins, Pacific ciguatoxins, Detection methods, High performance liquid chromatography, Tandem ion-spray mass spectrometry.

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Ciguatera (fish poisoning) is a major problem throughout tropical and sub-tropical waters with an estimated 25,000 persons poisoned annually. The disease is characterised by a constellation of neurological and gastrointestinal disorders which typically appear from 1 to 24 h following the consumption of contaminated fish (Gillespie *et al.*, 1986). The toxins involved are potent sodium channel activator toxins known as ciguatoxins that are produced by the benthic dinoflagellate *Gambierdiscus toxicus* (Lewis and Holmes, 1993). Two related families of ciguatoxins have recently been identified in fish. One family of toxins are from the Pacific (P-CTX) (Murata *et al.*, 1990; Lewis *et al.*, 1991; Pauillac *et al.*, 1995; Lewis and Jones, 1997) and the other from the Caribbean (C-CTX) (Crouch *et al.*, 1995; Vernoux and Lewis, 1997). The ciguatoxins are heat stable polyether toxins of 1023-1157 Da which accumulate to levels above 0.1 ppb in the flesh of ciguateric fish. In this report we describe the application of HPLC/MS methods for the identification of multiple ciguatoxins and HPLC/MS/MS methods for the detection of low levels of P-CTX-1 and C-CTX-1 in crude fish extracts.

## MATERIALS AND METHODS

### HPLC/mass spectrometry (HPLC/MS) identification of multiple ciguatoxins

Highly toxic lipid extracts of central Pacific Ocean moray eels (*Gymnothorax javanicus*) were purified on a silica gel, two Sephadex LH-20 and a TSK HW-40S column (Lewis *et al.*, 1991; Lewis and Jones, 1997). The Pacific ciguatoxins were then separated on a 5  $\mu$ m reverse phase PRP-1 HPLC column (0.41 x 15 cm; Hamilton) eluted at 0.5 ml/min with 50% B in A for 10 min, a linear gradient from 50-100% B over 20 min, and 100% B for a further 20 min (A = 0.1% TFA; B = 95% acetonitrile/0.1% TFA). The eluant was split and 40  $\mu$ l/min directed into a PE-Sciex (Toronto, Canada) atmospheric pressure ionisation (API) triple quadrupole MS equipped with a pneumatically assisted electrospray (Ionspray) interface (Lewis *et al.*, 1994). Positive ions were detected at an orifice potential (OR) of 70 V over the mass range  $m/z$  900-1200 at 0.1 Da steps.

A similar procedure as above was used to enrich ciguatoxins in carnivorous fishes from the Caribbean Sea (Vernoux and Lewis, 1997) and an aliquot applied to a Vydac 201 HS (250 x 2.1 mm) column eluted at 130  $\mu$ l/min with a linear 1% gradient of from 50%-100% B in A (A = 10% acetonitrile/0.1% TFA; B = 100% acetonitrile/0.09% TFA) and 100% B for 10 min. Positive ions ( $m/z$  700-1300) were monitored by ionspray MS at OR = 70 V, with turbo  $N_2$  flowing at 5 l/min and 550°C. MacSpec 3.3 and MacBiospec were used to process these data. Spectra from the HPLC/MS were generated without baseline subtraction.

### HPLC/MS/MS detection of P-CTX-1 and C-CTX-1 in crude extracts of fish

Using pure P-CTX-1, C-CTX-1 and PbTx-2 as reference compounds, MS/MS conditions were established that maximised signals from at least two ion fragments originating in each case from the  $[M + NH_4]^+$  ion (Lewis *et al.*, 1998). Serial dilutions of a stock solution containing P-CTX-1 (Lewis *et al.*, 1991), C-CTX-1 (Vernoux and Lewis, 1997) and PbTx-2 each at 10  $\mu$ g/ml were analysed by HPLC/MS/MS optimised to detect P-CTX ( $m/z$  1128.7  $\rightarrow$  1094.0; 1128.7  $\rightarrow$  1076.0; 1128.7  $\rightarrow$  1058.0), C-CTX-1 (1158.6  $\rightarrow$  1123; 1158.6  $\rightarrow$  1105.5), and PbTx-2 (912.5  $\rightarrow$  895.5; 912.5  $\rightarrow$  877.5) at an orifice potential of

80 V. The eluant was delivered into a turbo-assisted MS with turbo  $N_2$  flowing at 5 l/min and 550°C. In addition, portions of the flesh of 30 fish from the Caribbean were extracted and partially cleaned on a Florisil column as described by Vernoux and Lewis (1997) and their potency (non-toxic, borderline, toxic) determined by mouse bioassay (Vernoux, 1994). P-CTX-1 was spiked into these extracts at 1-6 ng per injection as an internal standard. To determine the extent of any matrix effect on the HPLC/MS/MS response, serial dilutions of this stock were spiked into 2.5 g of flesh extracts of two non-toxic fish. Samples were stored at -20°C and dilutions prepared in 70% acetonitrile. For HPLC/MS/MS analysis of these samples, 5 µl aliquots of extracts from 50 g fish dissolved in 100 µl of 70% acetonitrile were tested. HPLC conditions comprised a 5 µm Vydac 201 HS (250 x 2.1 mm) column eluted at 150 µl/min with 50% A for 2 min, a linear 2% gradient of from 50%-100% B in A, and 10 min at 100% B (A = 0.05% TFA; B = 90% acetonitrile/0.05% TFA).

## RESULTS AND DISCUSSION

### HPLC/MS detection of multiple ciguatoxins in Pacific fish

Gradient reverse phase HPLC/MS was used to identify Pacific and Caribbean ciguatoxins in partially purified extracts from fish (Fig. 1). Figure 1A identifies C-CTX-1 and C-CTX-2 as the major ciguatoxins present in a partially purified extract of horse-eye jack, with the spectra for two earlier eluting compounds shown as insets. These latter molecules ( $[M + H]^+$   $m/z$  1157.6 and 1143.6) are likely to be ciguatoxins since they readily formed ammonium ions and lost water (Vernoux and Lewis, 1997). Figure 1B identifies P-CTX-1, -2 and -3 as the major ciguatoxins present in an enriched extract from moray eel on the basis of mass and retention time. Four minor ciguatoxins were also detected under these conditions, with each giving rise to both  $[M + NH_4]^+$  and  $[M + Na]^+$  ions and ions associated with the loss of waters (Lewis and Jones, 1997). The inset in figure 1B is likely to be either a less polar variant of P-CTX-1 or a more polar variant of P-CTX-2 and/or P-CTX-3. These minor components could be detected in the P-CTX enriched fraction from moray eel viscera to levels equivalent to ~ 0.3 ppb but were not detected by mouse bioassay. Thus gradient reverse phase HPLC/MS provides a flexible and relatively sensitive approach to the identification of known and new ciguatoxins. Unfortunately, even with a multi-step sample clean-up procedure the sensitivity of the approach is not sufficient to detect ciguatoxins in low toxicity ciguateric fish. In fact, the sensitivity of this method deteriorated when crude extracts were tested. This lack of sensitivity was overcome with the HPLC/MS/MS method described below.

### HPLC/MS/MS detection of P-CTX-1 and C-CTX-1 in crude extracts of fish

MS/MS conditions were established to maximise the signal for at least two fragment ions from P-CTX-1, C-CTX-1 and PbTx-2. An example of the HPLC/MS/MS responses obtained for 1.0 ng P-CTX-1, C-CTX-1 and PbTx-2 (Fig. 2A) and for 1 ng of each toxin spiked into 2.5 g of fish extract (Fig. 2B). Similar responses were obtained in the presence of crude fish extract, with small differences in retention due to slight differences in the column equilibration for each run. HPLC analysis of responses to pure P-CTX-1, C-CTX-1 and PbTx-2 at levels from 0.1 to 50 ng per injection indicated that the response to each polyether was linear. Spiking these toxins into a 2.5 g of fish extract also gave

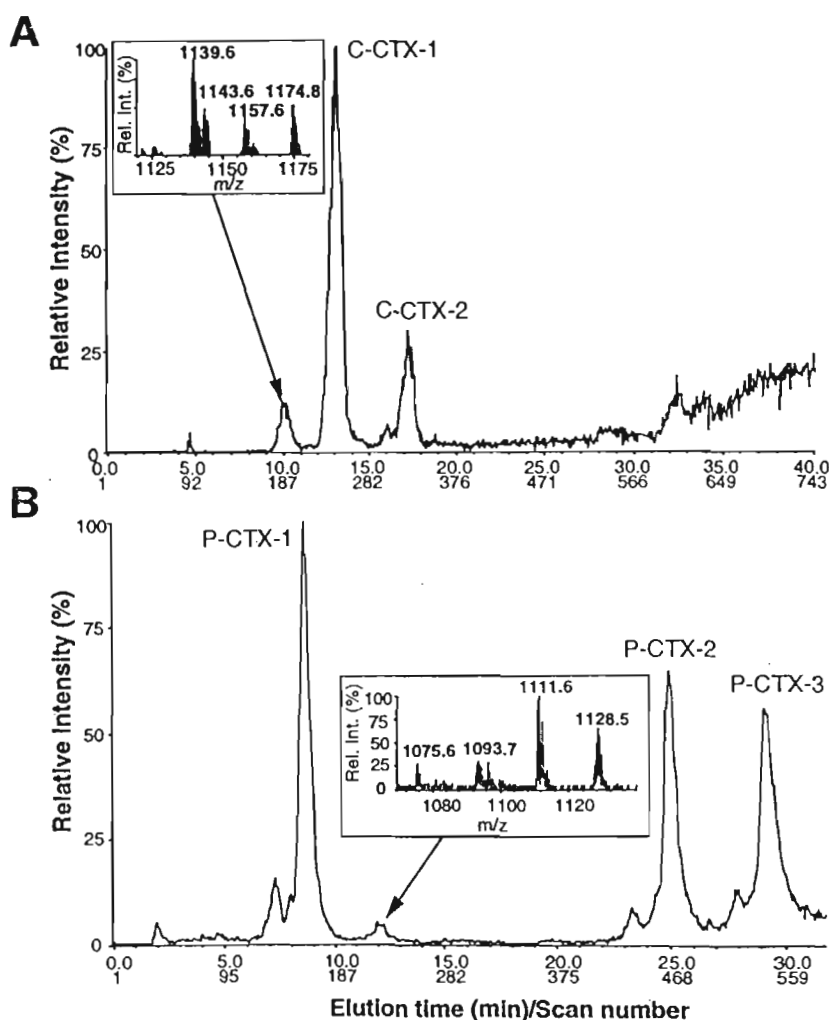


Fig. 1. - Gradient reverse phase HPLC/MS of (A) Caribbean ciguatoxins and (B) Pacific ciguatoxins. (A) Partially purified extract from Caribbean horse-eye jack was analysed, with data from the  $m/z$  1090-1150 window shown. (B) Partially purified extract from Pacific moray eel was analysed, with data from the  $m/z$  1100-1160 window shown. Insets show spectra for selected uncharacterised ciguatoxin-like compounds found in Caribbean and Pacific fish.

linear responses, but responses for P-CTX-1, C-CTX-1 and PbTx-2 were reduced by 15%, 37% and 58% in the presence of fish extract. The minimum detection level for P-CTX-1 and C-CTX-2 spiked into this crude fish extract was estimated to be 0.1 and 0.5 ng, giving detection limits of 0.04 and 0.2 ppb in fish flesh, respectively. These levels of sensitivity are believed to be sufficient to characterise ciguatoxins contaminating ciguateric fish of Pacific and Caribbean origin.

Using P-CTX-1 as an internal standard, the levels of C-CTX-1 present in crude fish extracts for 30 Caribbean fish was determined (Lewis *et al.*, in press). These results indi-

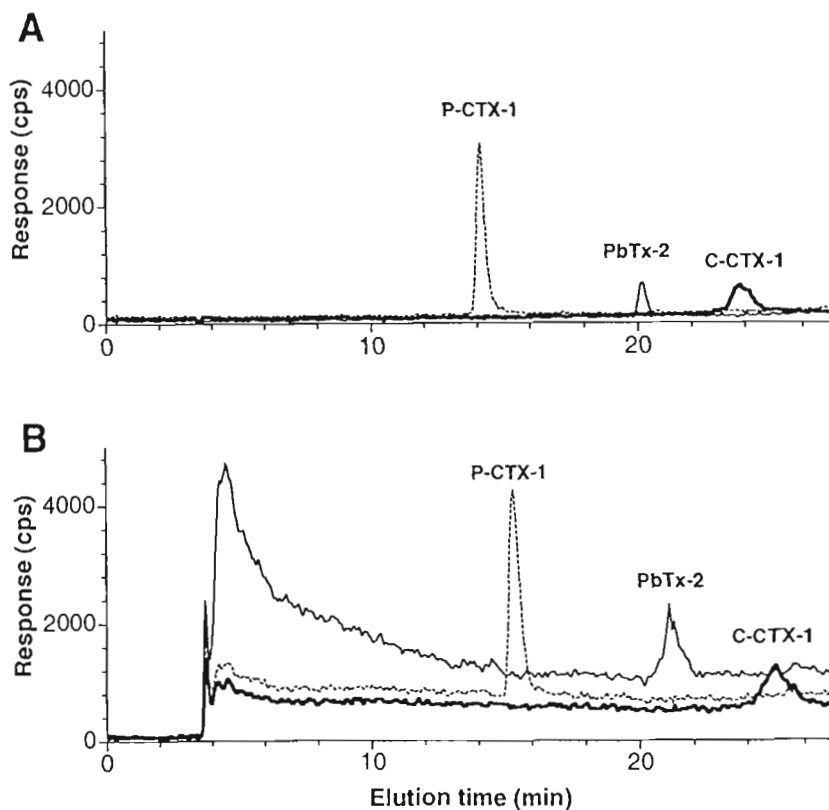


Fig. 2. - Two examples of HPLC/MS/MS detection of polyether toxins. (A) Responses to 1.0 ng P-CTX-1, PbTx-2 and C-CTX-1. (B) Responses to 1.0 ng P-CTX-1, PbTx-2 and C-CTX-1 spiked into an extract of 2.5 g of non-toxic fish flesh.

cated that (i) all fish identified as toxic by the mouse bioassay contained  $> 0.7$  ppb C-CTX-1 (ii) only 2 of the 14 non-toxic fish contained detectable C-CTX-1 and (iii) 8 of the 12 borderline toxic fish contained detectable C-CTX-1. The most toxic fish was found to contain 2.5 ng C-CTX-1 per g fish flesh. Mouse bioassay *vs* HPLC/MS/MS data produced a linear relationship that indicated  $\sim 50\%$  of the toxicity of Caribbean fish can be attributed to C-CTX-1. Previous studies have identified multiple toxins contributing to toxicity in ciguateric fish of the Caribbean (Vernoux and Talha, 1989; Vernoux and Andaloussi, 1986; Vernoux and Lewis, 1997). The number of toxins contributing to ciguatera in the Caribbean Sea and how these toxins induce human ciguatera that is slightly different to ciguatera in the Pacific remains to be determined. Previous analytical methodology for detecting P-CTX-1 have only proved useful to detect pure ciguatoxin and no rapid assay for the ciguatoxins has been developed (Pauillac *et al.*, 1995) although antibody, sodium channel binding and cell-based assays hold much promise. The HPLC/MS/MS method reported here has the sensitivity and flexibility to be used as an analytical method to identify the major toxins contributing to ciguatera in the Pacific and Caribbean. Rapid

extraction and HPLC methods that take advantage of the sensitivity of HPLC/MS/MS are now being developed in our laboratories.

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## IONIC BASIS OF THE NEUROCELLULAR ACTIONS OF PACIFIC CIGUATOXINS IMPLICATED IN CIGUATERA FISH POISONING

by

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**ABSTRACT.** - Ciguatoxins are responsible for a human seafood poisoning named ciguatera, a disease linked to the benthic dinoflagellate *Gambierdiscus toxicus* and acquired by eating certain contaminated fish species. These toxins are complex, lipid-soluble, cyclic polyethers which bind with high affinity to a specific receptor site of the neuronal, voltage-sensitive  $\text{Na}^+$  channel-protein. Pharmacological studies reveal that ciguatoxins increase  $\text{Na}^+$  permeability of various excitable cell membranes, notably at the resting membrane potential. This action is attributed to modification of  $\text{Na}^+$  channels, which then remain permanently open. As a consequence, ciguatoxins evoke membrane depolarization, cause spontaneous and/or repetitive action potentials, and influence  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchange in nerve membranes. Moreover, they induce mobilization of intracellular  $\text{Ca}^{2+}$  in nerve cells. Finally, ciguatoxins produce swelling of nerve cells due to continuous  $\text{Na}^+$  entry through toxin-opened  $\text{Na}^+$  channels, which induces an increase in intracellular  $\text{Na}^+$  concentration and an influx of water. These latter effects are prevented by blocking voltage-dependent  $\text{Na}^+$  channels and are reversed by hyperosmolar external solutions containing, in particular, D-mannitol. In conclusion, these neurocellular actions may explain some of the human neurological alterations induced by ciguatoxins and the efficacy of D-mannitol used as a clinical treatment of ciguatera.

**RÉSUMÉ.** - Bases ioniques des actions neurocellulaires des ciguatoxines du Pacifique.

Les ciguatoxines sont responsables d'un empoisonnement marin humain, appelé ciguatera, une maladie liée au dinoflagellé benthique *Gambierdiscus toxicus* et contractée en mangeant certaines espèces de poissons contaminés. Ces toxines sont des polyéthers cycliques complexes, liposolubles, qui se fixent avec une forte affinité sur un site récepteur spécifique de la protéine-canal  $\text{Na}^+$  neuronale sensible au potentiel. Des études pharmacologiques révèlent que les ciguatoxines augmentent la perméabilité au  $\text{Na}^+$  de diverses membranes cellulaires excitables, notamment au potentiel de repos de la membrane. Cette action est attribuée à la modification de canaux  $\text{Na}^+$  qui restent ouverts en permanence. En conséquence, les ciguatoxines induisent une dépolarisation membranaire, provoquent des potentiels d'action spontanés et/ou répétitifs et activent l'échangeur  $\text{Na}^+$ - $\text{Ca}^{2+}$  dans les membranes nerveuses. De plus, elles induisent la mobilisation du  $\text{Ca}^{2+}$  intracellulaire dans les cellules nerveuses. Finalement, les ciguatoxines produisent un gonflement des cellules nerveuses dû à l'entrée continue de  $\text{Na}^+$  au travers des canaux  $\text{Na}^+$  ouverts par les toxines, ce qui induit une augmentation de la concentration intracellulaire de  $\text{Na}^+$  et un influx d'eau. Ces derniers effets ne se produisent pas lorsque les canaux  $\text{Na}^+$  dépendants du potentiel sont bloqués, et sont réversés par des solutions externes hyperosmotiques contenant, en particulier, du D-mannitol. En conclusion, ces actions neurocellulaires peuvent expliquer certaines altérations neurologiques humaines induites par les ciguatoxines et l'efficacité du D-mannitol utilisé dans le traitement clinique de la ciguatera.

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Key-words. - Ciguatera fish poisoning, Ciguatoxins, D-mannitol, Myelinated axons, Motor nerve terminals, Neuroblastoma cells, Synaptosomes, Electrophysiology, Confocal laser scanning microscopy, Action potential, Voltage-dependent  $\text{Na}^+$  channels,  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchange, Intra-cellular  $\text{Ca}^{2+}$ , Cellular volume.

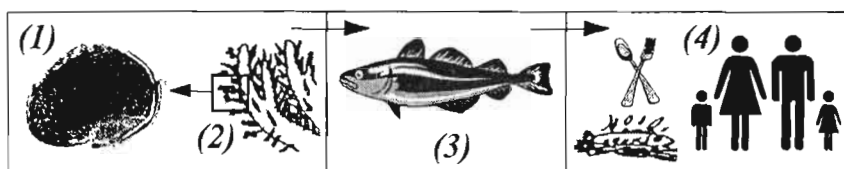


Fig. 1. - Transmission path of ciguatoxins along the marine food chain. The toxins originate in the marine dinoflagellate *Gambierdiscus toxicus* (1) associated with algae on the surface of dead corals (2), and are transmitted through herbivorous and carnivorous fish (3) to man (4).

Ciguatoxins are responsible for a distinctive and common form of a widespread, complex, human seafood intoxication known as ciguatera fish poisoning, characterized by severe gastrointestinal and neurological disturbances which develop after consumption of certain contaminated species of tropical and subtropical coral reef fishes (Gillespie *et al.*, 1986; Anderson and Lobel, 1987; Russell and Egen, 1991; Swift and Swift, 1993; Yasumoto and Murata, 1993; Glaziou and Legrand, 1994). Ciguatera fish poisoning has been linked to the toxic benthic dinoflagellate *Gambierdiscus toxicus* (Adachi and Fukuyo, 1979), since its discovery in the Gambier Islands in French Polynesia (Bagnis *et al.*, 1977; Yasumoto *et al.*, 1977). The dinoflagellate is believed to elaborate the toxins which are transmitted to fish through the marine food chain and ultimately to man (reviewed by Anderson and Lobel, 1987; Russell and Egen, 1991; Lewis and Holmes, 1993; Swift and Swift, 1993; see Fig. 1).

### Chemical structure of ciguatoxins

Ciguatoxins are a family of complex, lipid-soluble, highly oxygenated, cyclic polyethers (Scheuer *et al.*, 1967; Tachibana *et al.*, 1987; Legrand *et al.*, 1989; Murata *et al.*, 1989, 1990; Lewis *et al.*, 1991; Lewis and Sellin, 1992; Scheuer, 1994). Several ciguatoxins have been extracted and highly purified from biodetritus containing wild *Gambierdiscus toxicus* (Legrand *et al.*, 1992; Murata *et al.*, 1990; Holmes *et al.*, 1991) or from cultured dinoflagellate strains isolated from different areas of the world (Holmes *et al.*, 1991) and from various poisonous fish (Legrand *et al.*, 1989, 1992; Lewis and Sellin, 1992). The chemical structures of various ciguatoxins from poisonous Pacific fish and *G. toxicus* are known, including CTX (= CTX-1B or CTX-1 and considered the major ichthyotoxin involved in ciguatera fish poisoning), CTX-4B, CTX-2 and CTX-3 (Murata *et al.*, 1989, 1990; Lewis *et al.*, 1991; see also Fig. 2). Recently, ciguatoxins extracted from Caribbean fish were characterized (Vernoux and Lewis, 1997). Although their chemical structure remains to be determined, these toxins are reported to be distinct chromatographically from those found in Pacific fish, which indicates that they are members of a new family of ciguatoxins. Ciguatoxins have a chemical structure similar to that of the brevetoxins (PbTx-n), another family of potent lipid-soluble polyethers purified to homogeneity from laboratory cultures of the marine dinoflagellate *Ptychodiscus brevis* (formerly *Gymnodinium breve*; Davis, 1947), which include PbTx-1 to PbTx-10 (Baden,



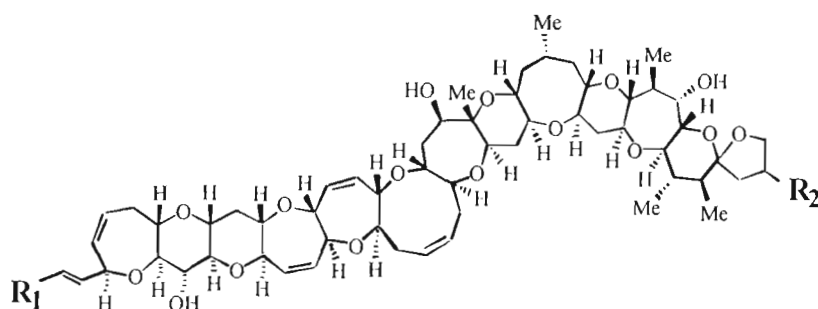


Fig. 2. - Chemical structures of CTX-1B purified from the moray-eel *Gymnothorax javanicus*:  $R_1 = -CH(OH)-CH_2OH$ ,  $R_2 = OH$ , and of CTX-4B purified from the dinoflagellate *Gambierdiscus toxicus*:  $R_1 = -CH=CH_2$ ,  $R_2 = H$ . Modified after Murata *et al.*, 1990.

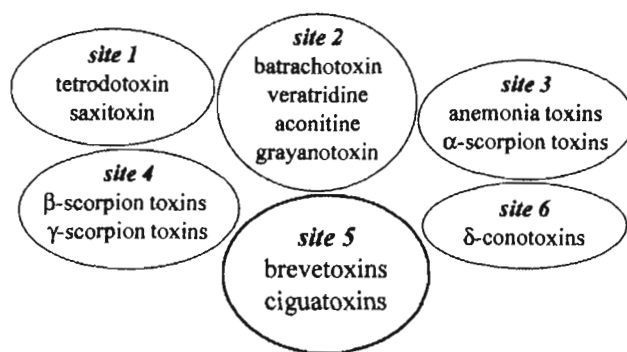


Fig. 3. - Toxin receptor sites identified on the neuronal, voltage-dependent  $Na^+$  channel-protein.

1989; Gawley *et al.*, 1992). It is worth noting that brevetoxins have been implicated, during blooms of *P. brevis* (red tides), in episodes of massive fish kills and human intoxications (Baden, 1989). Chemical structural evidence suggests that CTX-1B, extracted from the moray-eel *Gymnothorax javanicus*, results from the oxidative modification of CTX-4B produced by the dinoflagellate *Gambierdiscus toxicus* (see Murata *et al.*, 1990; Fig. 2).

### Ciguatoxins bind to a specific receptor site of neuronal, voltage-sensitive $Na^+$ channels

In addition to having similar chemical structures, ciguatoxins and brevetoxins share a common and specific binding site on the neuronal, voltage-sensitive  $Na^+$  channel-protein (Lombet *et al.*, 1987; Baden, 1989; Lewis *et al.*, 1991; Gawley *et al.*, 1992; Pauillac *et al.*, 1995). More than 12 distinct classes of biologically-active neurotoxins interact with at least 6 specific receptor sites identified on the neuronal,  $Na^+$  channel-protein by binding studies (Fig. 3). Brevetoxins bind with high affinity to the neurotoxin receptor site 5 of  $Na^+$  channels, as revealed by direct binding studies using radiolabeled [ $^3H$ ]PbTx-3 and by binding assays involving competition of brevetoxins with a variety of toxin probes specific for sites 1-4 of  $Na^+$  channels (Bidard *et al.*, 1984; Poli *et al.*, 1986; Sharkey *et al.*, 1987). Ciguatoxins are not available in sufficient amounts to prepare radiolabeled compounds for direct investigation of their specific binding; therefore,

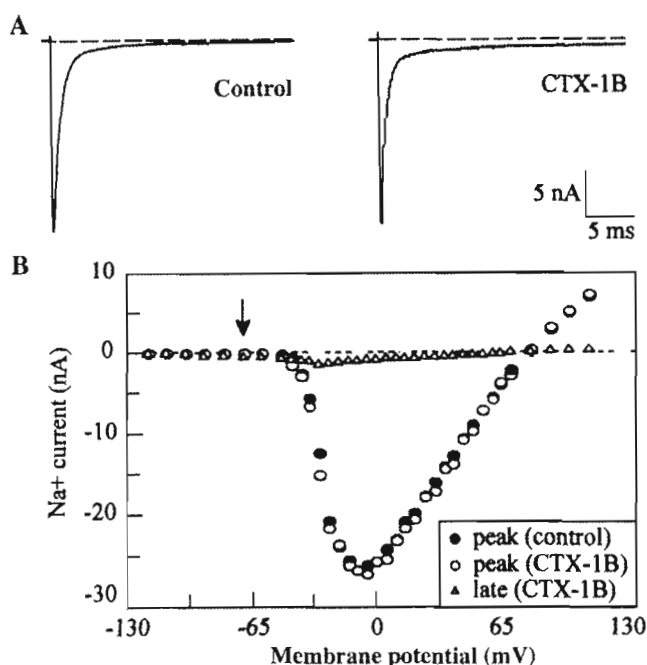


Fig. 4. - Effects of CTX-1B on the nodal Na<sup>+</sup> current of myelinated axons. The current was recorded under voltage-clamp conditions during depolarizing steps to 0 mV (A) or at various amplitudes (B) preceded by 50 ms hyperpolarizations to -120 mV applied at 0.5 Hz from a -70 mV holding potential. In A, the interrupted lines indicate the zero current level. (A) Traces of Na<sup>+</sup> current in the absence (left) and in the presence (right) of 10 nM CTX-1B. (B) Na<sup>+</sup> current-voltage relationships before (filled symbols) and after (open symbols) addition of 10 nM CTX-1B to the standard physiological solution. Peak Na<sup>+</sup> current (circles) and late Na<sup>+</sup> current (triangles) were measured during and at the end of depolarizations, and were plotted against the membrane potential. In B, the arrow indicates -70 mV. Modified after Benoit *et al.*, 1996.

[<sup>3</sup>H]PbTx-3 has been used in homologous and heterologous displacement experiments. Competition of a purified ciguatoxic fraction (Lombet *et al.*, 1987) or the reference ciguatoxin CTX-1 (Lewis *et al.*, 1991) or CTX-1B (Pauillac *et al.*, 1995) with [<sup>3</sup>H]PbTx-3 strongly suggests that ciguatoxins and brevetoxins share the same specific binding site (site 5) on Na<sup>+</sup> channels.

Voltage-dependent Na<sup>+</sup> channels in various animal excitable tissues contain a major  $\alpha$ -subunit of about 240-280 kDa composed of about 2,000 amino acids organized in 4 repeated homologous domains (I to IV), each consisting of 6 putative transmembrane  $\alpha$ -helical segments (S1 to S6) (Gordon, 1990; Catterall, 1992). Using a photolabeled derivative of PbTx-3 and site-directed antibody mapping, the partial localization of the receptor site 5 of voltage-dependent Na<sup>+</sup> channels (from rat brain) has been suggested to be in the region of interaction of segments S6 and S5 of domains I and IV, respectively (Trainer, 1994).

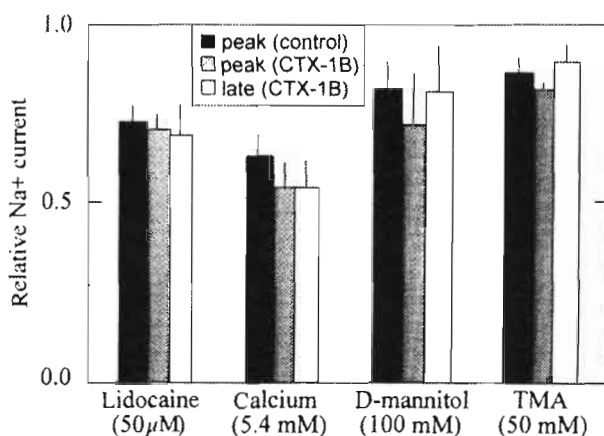


Fig. 5. - Effects of lidocaine (50  $\mu$ M), increased external  $\text{Ca}^{2+}$  concentration (from 1.8 to 5.4 mM), and increased external osmolality (by about 50%) with D-mannitol (100 mM) or tetramethylammonium (TMA, 50 mM) on unmodified (peak) and ciguatoxin-modified (late) nodal  $\text{Na}^+$  currents of frog myelinated axons. Under each condition, the peak  $\text{Na}^+$  current (black and gray bars) and the late  $\text{Na}^+$  current (white bars) were measured during and at the end of depolarizations to 0 mV from a holding potential of -70 mV, and were normalized to respective values before addition of the various agents to the external solution. Data represent the mean  $\pm$  SEM of 3-5 experiments.

### Ciguatoxins activate voltage-sensitive $\text{Na}^+$ channels in nerve membranes

Most of the pharmacological studies on the effects of ciguatoxins have been performed using a purified ciguatoxic fraction or a reference ciguatoxin; i.e., CTX, CTX-1B or CTX-1. These studies identify ciguatoxins as potent, voltage-dependent  $\text{Na}^+$  channel-activating toxins. They reveal that nanomolar concentrations of ciguatoxins, through an action on voltage-dependent  $\text{Na}^+$  channels, alter the properties of electrically excitable cell membranes in various tissues and thus elicit an increase in  $\text{Na}^+$  permeability, notably at the resting membrane potential (reviewed by Molgó *et al.*, 1992a).

The effects of CTX-1B, purified from the poisonous moray-eel *G. javanicus*, have been assessed on the nodal  $\text{Na}^+$  current of single myelinated axons (Benoit *et al.*, 1986, 1996; Benoit and Legrand, 1992). When myelinated axons were exposed to CTX-1B (0.44 to 10 nM), a fraction of the  $\text{Na}^+$  current, named the « late current », failed to inactivate and remained constant during long lasting depolarizations (Fig. 4A). It is worth noting that under control conditions the  $\text{Na}^+$  current fully inactivated during depolarizations. Neither the amplitude of the peak  $\text{Na}^+$  current nor its voltage-dependence were modified by CTX-1B in the range of concentrations studied (Fig. 4B). In contrast, the activation threshold potential, the potential corresponding to maximum inward current, and the reversal potential were more negative for the late  $\text{Na}^+$  current than for the peak  $\text{Na}^+$  current. Therefore, in the presence of CTX-1B, the late  $\text{Na}^+$  current was activated at the resting membrane potential of fibres (-70 mV; Fig. 4B). It was concluded that the neurocellular actions of ciguatoxins involve the modification of a fraction of  $\text{Na}^+$  channels, which remain permanently open (notably at the resting membrane potential) instead of being maintained in a closed state (Benoit *et al.*, 1986, 1996; Benoit and Legrand, 1992). Whether the  $\text{Na}^+$  channels which are responsible for the late  $\text{Na}^+$  current are a subset of channels distinct from those which are responsible for the peak  $\text{Na}^+$  current remains to be

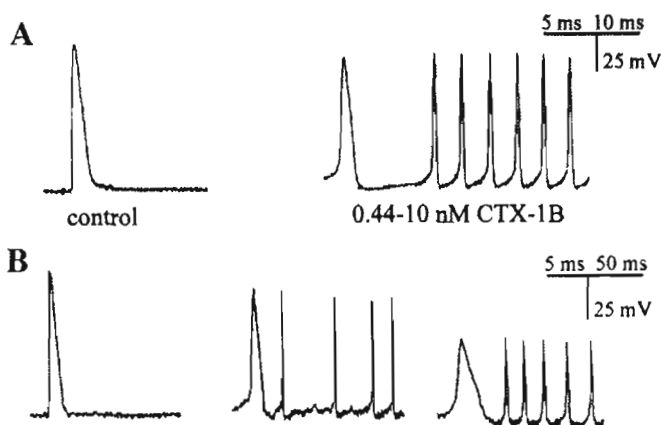


Fig. 6. - Effects of CTX-1B and CTX-4B on the nodal action potential of myelinated axons. Data were obtained by the conventional current-clamp technique. Under control conditions, action potentials were evoked by 0.5 ms depolarizing stimuli. After addition of either CTX-1B (A) or CTX-4B (B) to the standard physiological solution, spontaneous and repetitive action potentials are observed. Modified after Benoit and Legrand, 1994 and after Benoit *et al.*, 1996.

determined. Also, further experiments are needed to infer whether brevetoxins act in a similar manner.

Although it is clear that ciguatoxins affect the biophysical properties of a fraction of  $\text{Na}^+$  channels, some of the pharmacological properties of toxin-modified  $\text{Na}^+$  channels have been reported to remain unaffected (Benoit and Legrand, 1992; Benoit *et al.*, 1996). In particular, both peak  $\text{Na}^+$  current (i.e., unmodified  $\text{Na}^+$  channels) and late  $\text{Na}^+$  current (i.e., toxin-modified  $\text{Na}^+$  channels) were affected in a similar manner by the local anesthetic lidocaine, by increasing the external concentration of  $\text{Ca}^{2+}$  ions, and by increasing the external osmolality with D-mannitol, tetramethylammonium or sucrose (Fig. 5).

#### Ciguatoxins increase excitability of nerve membranes

As a consequence of the persistent activation of voltage-dependent  $\text{Na}^+$  channels at resting membrane potential, ciguatoxins increase excitability of nerve membranes. In particular, CTX-1B evokes membrane depolarization which in turn causes spontaneous and/or repetitive action potential discharges at high frequencies (60-100 Hz) in myelinated axons and motor nerve terminals (Benoit *et al.*, 1986, 1996; Molgó *et al.*, 1990, 1992a; Benoit and Legrand, 1992; see Fig. 6A). In neuroblastoma cells, CTX-1B was reported to induce a membrane depolarization and, under appropriate conditions, to create spontaneous oscillations in the membrane polarization level and repetitive action potentials (Bidard *et al.*, 1984).

Relatively little work has been done with CTX-4B from the dinoflagellate *Gambierdiscus toxicus*, mainly due to difficulties in obtaining purified toxin. However, preliminary studies revealed that the effects of CTX-4B on myelinated nerve fibres are similar to those of CTX-1B (Benoit and Legrand, 1994). CTX-4B also induces spontaneous action potentials, although it is about 50-fold less effective than CTX-1B (Fig. 6B). Therefore, CTX-4B is thought to act as a  $\text{Na}^+$  channel activator, as does CTX-1B. However, in contrast to CTX-1B, CTX-4B both decreases the amplitude and increases the duration of spontaneous action potentials as compared to controls. These results indicate that CTX-4B is

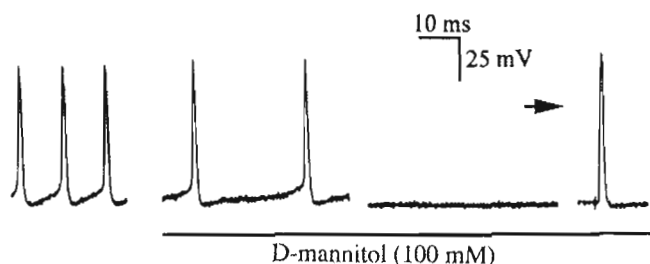


Fig. 7. - Hyperosmolar D-mannitol suppression of CTX-1B-induced nodal spontaneous and repetitive action potentials in myelinated axons. The addition of 100 mM D-mannitol to the external physiological solution containing CTX-1B (10 nM) suppressed spontaneous action potentials but did not affect action potentials evoked by depolarizing stimuli of 0.5 ms duration (arrow). Modified after Benoit *et al.*, 1996.

also a  $\text{Na}^+$  and  $\text{K}^+$  channel-blocking toxin. Thus, it appears that  $\text{Na}^+$  channel activation as well as  $\text{Na}^+$  and  $\text{K}^+$  channel blockade may be involved in the neurocellular actions of ciguatoxins.

It is worth noting that, in myelinated axons, the local anaesthetic lidocaine (50 mM), an increase in the external concentration of  $\text{Ca}^{2+}$  (from 1.8 to 5.4 mM), or an increase in external osmolality with D-mannitol (100 mM), tetramethylammonium chloride (50 mM) or sucrose (100 mM) first decrease the frequency of spontaneous and repetitive action potentials induced by either CTX-1B or CTX-4B, and then progressively suppress the action potentials (Benoit and Legrand, 1992, 1994; Benoit *et al.*, 1996; see Fig. 7). However, under these various conditions, action potentials evoked by depolarizing stimuli could still be obtained. Therefore, lidocaine or an increase in either external concentration of  $\text{Ca}^{2+}$  ions or external osmolality reduces the membrane excitability previously increased by ciguatoxins to a level similar to that under control conditions, i.e., reverses the effects of ciguatoxins.

The ability of ciguatoxins to increase membrane excitability is consistent with studies on nerve conduction showing that partially purified preparations of the toxins, although increase and prolong the supernormal period of the nerve excitability curve (Flowers *et al.*, 1988; Cameron *et al.*, 1991a, 1991b). Moreover, ciguatoxins-produced membrane depolarization and increased excitability of axons and nerve terminals are responsible for numerous effects in a variety of chemical synapses and secretory terminals (reviewed by Molgó *et al.*, 1992a, 1998). In particular, ciguatoxins (i) first transiently enhance synaptic transmission by producing spontaneous or repetitive synchronous neurotransmitter release and by increasing the mean quantal content of the synaptic responses, (ii) subsequently reduce and finally block synchronous neurotransmitter release irreversibly, probably due to nerve terminal depolarization which prevents action potential generation, and (iii) greatly increase spontaneous quantal asynchronous neurotransmitter release. These alterations of synaptic transmission mechanisms caused by ciguatoxins are directly related to the activation of  $\text{Na}^+$  channels and the consequent entry of  $\text{Na}^+$  ions into the nerve terminals, since they are either prevented or reversed by tetrodotoxin (which blocks voltage-dependent  $\text{Na}^+$  channels).

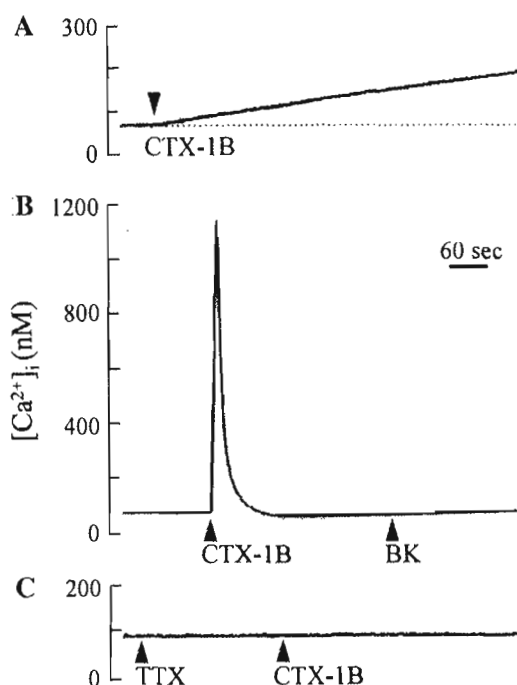


Fig. 8. - Mobilization of intracellular  $Ca^{2+}$  concentration by CTX-1B (2.5 nM, A; 25 nM, B) and its inhibition by 1  $\mu$ M tetrodotoxin (TTX) (C) in differentiated NG108-15 neuroblastoma x glioma cells.  $Ca^{2+}$  levels were measured by microspectrofluorometry with the  $Ca^{2+}$ -sensitive probe Fura-2/AM. Notice the sustained (A) and transient (B) increase in the intracellular  $Ca^{2+}$  concentration caused by 2.5 and 25 nM CTX-1B, respectively, and the CTX-1B (25 nM) inhibition of the subsequent action of 1  $\mu$ M bradykinin (BK). Arrows indicate addition of drugs to the nominally  $Ca^{2+}$ -free medium supplemented with 1 mM EGTA. Modified after Molgó *et al.*, 1993.

### Ciguatoxins induce mobilization of intracellular $Ca^{2+}$ in nerve cells

Nanomolar concentrations of CTX-1B (2.5 to 25 nM) increase the intracellular  $Ca^{2+}$  concentration in cultured, differentiated NG108-15 neuroblastoma x glioma hybrid cells, as determined with the fluorescent probe fura-2/AM (Molgó *et al.*, 1992b, 1993b). The increase occurred either in cells bathed in a standard medium containing  $Ca^{2+}$  or after exposure to a  $Ca^{2+}$ -free medium supplemented with the  $Ca^{2+}$  chelator EGTA (Fig. 8A, B). Tetrodotoxin prevented the CTX-1B-induced increase in intracellular  $Ca^{2+}$  concentration (Fig. 8C), which suggests that  $Ca^{2+}$ -mobilization depends on  $Na^+$  influx through voltage-dependent  $Na^+$  channels (Molgó *et al.*, 1992b, 1993b). These findings, if extended to include motor nerve terminals, could explain the increase in spontaneous quantal neurotransmitter release reported at neuromuscular junctions exposed to a  $Ca^{2+}$ -free medium supplemented with EGTA (Molgó *et al.*, 1990, 1991).

CTX-1B-induced  $Ca^{2+}$  mobilization prevented the subsequent action of bradykinin (Fig. 8B), which suggests that the intracellular  $Ca^{2+}$  store stimulated by CTX-1B is the same as the one activated by bradykinin (Yano *et al.*, 1984); i.e., the inositol 1,4,5-trisphosphate ( $IP_3$ )-releasable  $Ca^{2+}$  store (see reviews by Henzi and Macdermott, 1992; Kostyuk and Verkhatsky, 1994). The possibility that the inhibition of bradykinin-

induced increase in intracellular  $\text{Ca}^{2+}$  concentration results from competition between CTX-1B and bradykinin was rejected because it is difficult to reconcile with the dependence of CTX-1B on  $\text{Na}^+$  influx (Molgó *et al.*, 1992b, 1993b). Substantial evidence indicates that enhanced influx of  $\text{Na}^+$  can stimulate the production of  $\text{IP}_3$  in synaptoneurosome (Gusovsky *et al.*, 1986, 1987; Carrasco *et al.*, 1996) and in cardiac cells (McDonough *et al.*, 1988), presumably by activation of phospholipase C. Whether CTX-1B causes a similar increase in  $\text{IP}_3$  is not yet known and remains to be determined.

### **Ciguatoxins perturb $\text{Na}^+\text{-Ca}^{2+}$ exchange in nerve membranes**

In addition to activating voltage-dependent  $\text{Na}^+$  channels, ciguatoxins affect the operation of the  $\text{Na}^+\text{-Ca}^{2+}$  exchange system that normally uses the  $\text{Na}^+$  gradient to extrude  $\text{Ca}^{2+}$  ions.  $\text{Na}^+\text{-Ca}^{2+}$  exchange has been shown to be a completely electrogenic transport reaction in which the operation of the carrier is controlled by transmembrane ionic gradients and electric potentials. Thus, depending on prevailing ionic conditions,  $\text{Na}^+\text{-Ca}^{2+}$  exchange can act either as a  $\text{Ca}^{2+}$  efflux pathway or promote a net  $\text{Ca}^{2+}$  influx.

Exposure of cholinergic synaptosomes, isolated from the electric organ of the fish *Torpedo marmorata*, to CTX-1B (0.1 pM - 10 nM) revealed that the toxin increases  $\text{Na}^+$  influx into synaptosomes, thus favoring acetylcholine release triggered by  $\text{Ca}^{2+}$  (Molgó *et al.*, 1993a). This action does not seem to result from CTX-1B-induced depolarization of the synaptosomal membrane to levels above those needed to activate  $\text{Ca}^{2+}$  channels, because simultaneous blockade of  $\text{Ca}^{2+}$  channel subtypes by  $\text{Gd}^{3+}$ ,  $\omega$ -conotoxin GVIA, and FTX (a low molecular weight toxin purified from the venom of the American funnel-web spider *Agelenopsis aperta*) did not prevent acetylcholine release caused by CTX-1B upon addition of  $\text{Ca}^{2+}$ . In addition, almost no transmitter release was detected when  $\text{Na}^+$  was replaced by  $\text{Li}^+$ , which is consistent with the fact that  $\text{Li}^+$  can not replace  $\text{Na}^+$  in the  $\text{Na}^+\text{-Ca}^{2+}$  exchange process (Hermoni *et al.*, 1987). Furthermore, bepridil and ceticidil (inhibitors of the  $\text{Na}^+\text{-Ca}^{2+}$  exchange) completely prevented the  $\text{Ca}^{2+}$ -dependent acetylcholine release induced by CTX-1B (Morot-Gaudry-Talarmin *et al.*, 1996). Therefore, CTX-1B increases  $\text{Na}^+$  levels in synaptosomes and activates  $\text{Na}^+\text{-Ca}^{2+}$  exchange in the reversed mode, which causes  $\text{Ca}^{2+}$  entry into cholinergic synaptosomes in exchange for  $\text{Na}^+$  and, thereby, triggers  $\text{Ca}^{2+}$ -dependent acetylcholine release.

### **Ciguatoxins induce swelling of nerve cells**

Another consequence of the persistent activation of voltage-dependent  $\text{Na}^+$  channels induced by ciguatoxins at the resting membrane potential is the swelling of nerve cells. Swelling has been observed during the action of either CTX-1B or CTX-4B on myelinated axons, motor nerve terminals and on Schwann cells, using confocal laser scanning microscopy and the fluorescent dye FM1-43 to stain the plasma membrane of the preparations (Molgó *et al.*, 1994; Benoit *et al.*, 1996; Mattei *et al.*, 1997). CTX-4B induced a marked nodal swelling of single myelinated axons, without apparent modification of the morphology of the internodal parts of nerve fibres characterized by the presence of myelin sheath layers (Fig. 9A). Similarly, motor nerve terminals innervating skeletal muscle fibres, as well as perisynaptic Schwann cell somata, swelled when exposed to CTX-4B (Fig. 9B).

Both CTX-1B and CTX-4B caused about a two-fold increase in the nodal volume of myelinated axons and in motor nerve terminal area per unit length. The effect of the toxins on these two parameters could be decreased by increasing the osmolality of the external solution by about 50% with 100 mM D-mannitol (Fig. 10). Similar results (not shown) were obtained when the osmolality of the external solution was increased with

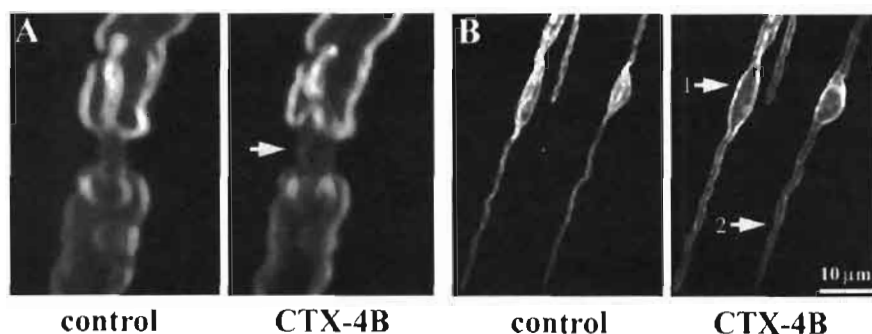


Fig. 9. - CTX-4B-induced swelling of nodes of Ranvier in myelinated axons and of motor nerve terminals *in situ*. The myelinated axon (A) and motor nerve terminals (B) were imaged before and 120 min after addition of either 24 nM (A) or 30 nM (B) CTX-4B to the standard physiological solution. Note the swelling of the node of Ranvier (arrow in A), of motor nerve terminals (arrow 2 in B) and of perisynaptic Schwann cell somata (arrow 1 in B). Confocal laser scanning microscopy was used for optical sectioning and high-resolution digital reconstruction of the three-dimensional structures. Modified after Mattei *et al.*, 1997.

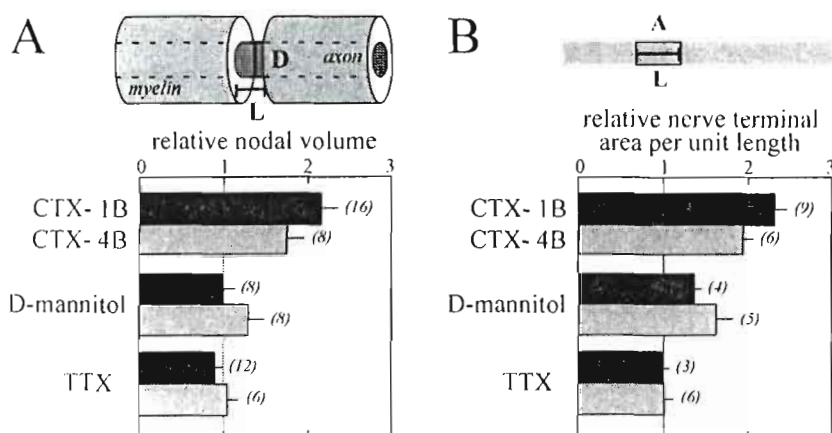


Fig. 10. - Quantification of the effects of ciguatoxins, tetrodotoxin, and hyperosmolar D-mannitol on nodes of Ranvier in frog myelinated axons (A) and on motor nerve terminals innervating frog skeletal muscle fibres (B). The upper part of A and B shows a schematic representation of a single myelinated axon (A) and of a motor nerve terminal (B). (A)  $L$  and  $D$  are the nodal length and diameter. The nodal volume ( $V$ ) was calculated assuming that the simplest geometry of a node of Ranvier approached a cylinder in which  $V = \pi L (D/2)^2$ . (B)  $A$  and  $L$  are the three-dimensional projected area and length of the nerve terminal. The nerve terminal area per unit length ( $A/L$ ) was calculated as an index of volume. In A and B, the histograms show the effects of CTX-1B (10 nM, black bars) and CTX-4B (24-30 nM, gray bars) before and after subsequent addition of D-mannitol (100 mM), and in the continuous presence of tetrodotoxin (1 mM, TTX). Parameters were normalized to control values and expressed as the mean  $\pm$  SEM. The numbers in parentheses denote the number of different preparations investigated.

tetramethylammonium chloride (50 mM) or with sucrose (100 mM). However, although the hyperosmolar external solutions almost completely reversed the ciguatoxins-induced



nodal swelling of myelinated axons, they only partially decreased toxin-induced swelling of motor nerve terminals (Fig. 10). Therefore, it appears that the action of ciguatoxins in increasing nerve terminal area per unit length is a consequence of the incorporation of synaptic vesicle membranes into the nerve terminal axolemma during stimulated neurotransmitter release, as well as osmotic changes (see Molgó *et al.*, 1994; Mattei *et al.*, 1997).

No significant change in the nodal volume of myelinated axons and in the motor nerve terminal area per unit length has been observed when the preparations were exposed to ciguatoxins in the continuous presence of the Na<sup>+</sup> channel blocker tetrodotoxin (Fig. 10). Thus, it has been proposed that ciguatoxins cause a continuous entry of Na<sup>+</sup> ions through (i) ciguatoxin-modified Na<sup>+</sup> channels permanently activated at the resting membrane potential and (ii) unmodified Na<sup>+</sup> channels which open during the ciguatoxin-induced spontaneous action potential discharges. This continuous Na<sup>+</sup> entry leads to an increase in intra-cellular Na<sup>+</sup> concentration that directly or indirectly disturbs the osmotic equilibrium between intra- and extracellular media. Therefore, an influx of water occurs in order to restore both the osmotic equilibrium and the intracellular Na<sup>+</sup> concentration to initial levels (see Molgó *et al.*, 1994; Benoit *et al.*, 1996; Mattei *et al.*, 1997).

## CONCLUSION

In conclusion, the neurocellular actions of ciguatoxins involve an increased Na<sup>+</sup> permeability of nerve membranes due to toxin-induced activation of voltage-dependent Na<sup>+</sup> channels, in particular at the resting membrane potential where these channels are normally maintained in a closed state. As a consequence, ciguatoxins affect various Na<sup>+</sup>-dependent mechanisms in nerve cells. In particular, they enhance membrane excitability, activate Na<sup>+</sup>-Ca<sup>2+</sup> exchange, induce mobilization of intracellular Ca<sup>2+</sup>, and produce cell swelling. These neurocellular actions of ciguatoxins are consistent with the generalized disturbance of nerve conduction, synaptic transmission and cellular morphology observed in intoxicated patients (Allsop *et al.*, 1986; Cameron *et al.*, 1991b), and may explain some of the human neurological alterations induced by these toxins. Furthermore, preliminary reports indicate that Na<sup>+</sup> and K<sup>+</sup> channel blockade, as well as Na<sup>+</sup> channel activation, may be involved in the neurocellular actions of ciguatoxins (Benoit and Legrand, 1994).

Treatment of ciguatera fish poisoning is primarily supportive. Herbal remedies are traditionally used in this treatment, and a list of about 100 plants reputedly active in the South Pacific has been reported (Laurent *et al.*, 1993). However, the basis for their efficacy as therapeutic agents remains to be determined. Although various other substances have been used for the treatment of ciguatera fish poisoning, such as local anaesthetics, calcium gluconate, vitamins, amitriptyline and glucose, it appears that intravenous hyperosmolar D-mannitol has evolved as a unique remedy for acutely poisoned patients (Palafox *et al.*, 1988; Pearn *et al.*, 1989; Russel and Egen, 1991; Blythe *et al.*, 1992). The basis for the beneficial action of D-mannitol in the treatment of ciguatera fish poisoning may be explained by its neurocellular actions: it decreases the ciguatoxin-induced increase in Na<sup>+</sup> permeability and excitability of nerve membranes, and shrinks nerve cells swollen by ciguatoxins.

**Acknowledgements.** - The work performed at the CNRS was funded by grants from Direction des Systèmes de Forces et de la Prospective (DSFP) and from International Cooperation Program of the

European Community. C.M. was supported by a fellowship from the DSFP. We thank A.S. Kreger, R.J. Lewis and M.A. Poli for helpful comments and careful reading of the manuscript.

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## **IMMUNOCHEMICAL METHODS FOR CIGUATOXINS DETECTION IN PACIFIC HERBIVOROUS AND CARNIVOROUS FISH**

by

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**ABSTRACT.** - A conjugate of JKLM ring fragment, a carboxylic derivative of the right-hand tetracyclic terminus portion of CTX-1B, the most potent ciguatoxin (CTX), with two carrier proteins has been synthesized. Hapten-carrier amide linkages were promoted using the N-hydroxysuccinimide ester of the carboxylic fragment in the presence of a water-soluble carbodiimide according to standard condensation procedure. Bovine serum albumin (BSA) and ovalbumin (OVA) were respectively used for i.p. immunization of two BALB/c mice and antibody screening. Titers of both antisera as determined in an end-point titration ELISA format were found to be around 1/16.000. Mice polyclonal antibodies (PABs) specificity was demonstrated either by indirect or by competitive inhibition ELISA. In the classical 96-well format, PABs did not significantly cross-react with PbTx-3 or other related polyether structures but did show 133% cross-reaction with CTX-1B. With a 1/30,000 antiserum dilution, the lower limit of accurate JKLM ring fragment and CTX-1B determinations was respectively 50 and 30 picomoles. Decreasing the antibody concentration (1/50.000 dilution), allowed a detection limit of 7 picomoles CTX-1B. The sensitivity of the assay could be further lowered to 0.6 picomoles JKLM fragment by means of a microELISA format performed in Terasaki plates. A preliminary assessment of CTXs in crude lipid extracts of fish yielded the same sensitivity (7 picomoles) as found for detection of pure CTX-1B performed in the standard ELISA format under the same conditions. It was concluded that the competitive ELISA described herein could be developed into a routine screening method for the determination of Pacific CTXs in crude fish extracts.

**RÉSUMÉ.** - Méthodes immunochimiques de détection des ciguatoxines dans les poissons herbivores et carnivores du Pacifique.

Un conjugué de l'albumine bovine (BSA) et de l'ovalbumine (OVA) avec un fragment synthétique JKLM de la CTX-1B, la plus puissante des ciguatoxines (CTXs), a été préparé. Les liaisons haptène-protéine porteuses ont été établies via le N-hydroxysuccinimide ester du fragment carboxylique, en présence d'un carbodiimide hydrosoluble. Les conjugués de la BSA et de l'OVA ont servi respectivement à l'immunisation de deux souris BALB/c et à l'analyse de leur réponse immunitaire par ELISA. Les titres en anticorps (Ac) mesurés par la technique de la dilution finale étaient autour de 1/16 000 dans les deux cas. La grande spécificité des Ac murins a été démontrée par ELISA de type indirect ou compétitif. Dans le format classique en plaques à 96 puits, les Ac n'ont pas réagi de manière significative avec la PbTx-3 et d'autres composés de nature polyéther mais ont présenté au contraire 133% de réaction croisée vis-à-vis de la CTX-1B. Des limites de détection pour JKLM et la CTX-1B de 50 et 30 picomoles respectivement ont été obtenues avec un antiserum dilué au 1/30 000. Une diminution de la concentration en Ac (dilution 1/50 000) a permis d'abaisser le seuil de détection à 7 picomoles de

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CTX-1B. La sensibilité de cette technique a pu encore être ramenée à 0,6 picomoles de fragment JKLM par utilisation d'un microtest ELISA réalisé en plaques de Terasaki. Un essai préliminaire d'évaluation de CTXs dans des extraits lipidiques bruts de poissons a fourni essentiellement la même sensibilité (7 picomoles) que lors des tests de détection de CTX-1B pure réalisés dans les mêmes conditions en plaques standard. En conclusion, le test ELISA compétitif décrit pourrait être amélioré pour permettre en routine le dosage direct des CTXs dans des extraits pisciaires.

Key-words. - Ciguatoxins, Antibody, ELISA, Micro-ELISA, Immunodetection.

Ciguatoxins (CTXs), a class of marine lipid-soluble polyether toxins produced by the benthic dinoflagellate *Gambierdiscus toxicus* are transferred from the benthos to herbivorous and then carnivorous coral reef fish via the marine food chain. These potent sodium channel activators are responsible for ciguatera, a human food poisoning endemic throughout the Caribbean and intertropical Indo-Pacific regions. The clinical picture is characterized by a variety of gastrointestinal, neurologic and occasionally cardiovascular symptoms (Bagnis *et al.*, 1979). Besides its obvious adverse effects on public health, ciguatera fish poisoning generates a negative impact on local fisheries, trade and tourism.

During the past decades the most commonly used detection tests for CTXs in fish tissues have been bioassays in such animals as mongoose, cat, chick, mouse, mosquito, brine shrimp and diptera larvae (reviewed by Park, 1994; Lewis, 1995). The disadvantages of these assays, combined with their moderate correlation at low CTXs level, have led to the development of a number of more practical and specific detection procedures based upon immunological, pharmacological and physico-chemical techniques (reviewed by Lewis, 1994, 1995; Park, 1994). However, in their present form these tests still require further improvements regarding specificity and/or sensitivity or necessitate the use of sophisticated and expensive instrumentation. In the course of designing a cost-effective method of detecting ciguateric fish prior to consumption, the development of enzyme- and radio-immunoassays (EIA and RIA) have been most actively pursued. Since 1977, Hokama and coworkers have reported the use of an RIA procedure with a sheep antiserum raised against partially purified CTX (probably CTX-1B) to distinguish toxic from non-toxic fishes (Hokama *et al.*, 1977). Tests for ciguateric fish have been developed based on RIA (Kimura *et al.*, 1982a, 1982b) and EIA (Hokama, 1985; Hokama *et al.*, 1983, 1984, 1987), and the use of monoclonal antibodies (MAbs) raised to CTX-1B or okadaic acid (OA) to improve specificity (Hokama, 1990; Hokama *et al.*, 1985, 1988, 1992). However, the reliability of such tests has been questioned (Berger and Berger, 1979; Laigret *et al.*, 1981; Chanteau *et al.*, 1981; Emerson *et al.*, 1983; Dickey *et al.*, 1994; Lewis, 1995), despite attempts at validation with field or clinically implicated fish samples (Hokama *et al.*, 1990, 1993; Ganai *et al.*, 1993). As a consequence, there is presently no adequately validated immunoassay method available for screening ciguateric fishes.

In this study we report our strategy towards the development of a laboratory antibody-based assay that could be scaled up to fit the requirement of a routine cost-effective mass screening of fishes prior to consumption. Due to the lack of pure CTXs, a conjugate of JKLM ring fragment (Sasaki *et al.*, 1994), a carboxylic derivative of the right-hand tetracyclic terminus portion of CTX-1B, the most potent CTX, (Murata *et al.*, 1989; Lewis and Sellin, 1992; Teai *et al.*, 1997) with two carrier proteins has been synthesized in bulk quantities. As it will be necessary to use tiny amount of CTX-1B or other congeners for immunization, a miniaturized conjugation procedure using PbTx-3, a member of the brevetoxins family, is also described.

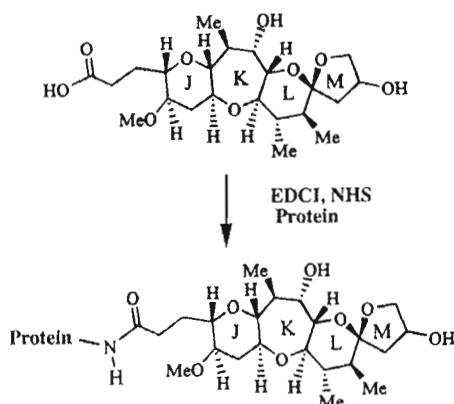


Fig. 1. - Chemical coupling of JKLM ring fragment to carrier proteins. Analysis of the conjugates by trinitrophenylation gave an epitope density of 16 hapten molecules per molecule of BSA and 10 per molecule of OVA. These conjugates were respectively used for mice immunization and for ELISA microtiter plates coating.

## MATERIALS AND METHODS

### Preparation of JKLM fragment-protein conjugate

The JKLM ring fragment used in this study has been synthesized as previously described by Sasaki *et al.* (1994). The purity of the haptenic carboxylic acid was checked by thin layer chromatography (TLC) using  $\text{CHCl}_3/\text{MeOH}$  (90/10) as developing solvent and 30% sulfuric acid as spraying reagent. After heating at  $120^\circ\text{C}$  a light brown spot ( $R_f = 0.2$ ) was observed with a yield greater than 80%. Further purification was performed on a Sep-Pak silica gel cartridge (Millipore) eluted with  $\text{CHCl}_3/\text{MeOH}$  (80/20). The pure carboxylic fragment came out immediately after the void volume. Samples of 3-5 mg of fragment were covalently conjugated to bovine serum albumin (BSA) to provide an efficient immunogen or to egg albumin (OVA) for antibody screening. Hapten-carrier amide linkages were promoted using the N-hydroxysuccinimide (NHS) ester of the carboxylic fragment in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) according to standard condensation procedure (Fig. 1). Following overnight incubation at room temperature under constant stirring, the reaction mixture was extensively dialyzed against 2 liters of deionized distilled water at  $4^\circ\text{C}$ , and lyophilized for preservation. The haptenic density of the conjugates was evaluated by measuring their remaining free amino groups according to Habeeb (1966).

### Immunization procedure

Two adult female BALB/c mice were each given one intraperitoneal (i.p.) injection of  $100\text{ }\mu\text{g}$   $(\text{JKLM})_{16}$ -BSA conjugate mixed 1:1 with Freund's complete adjuvant (total volume  $200\text{ }\mu\text{l}$ ) followed by 3 other injections, spaced 2 weeks apart, with Freund's incomplete adjuvant. Mice were sequentially bled from the retro-orbital venous system and their sera analyzed by indirect ELISA.

### Enzyme-linked immunosorbent assay (ELISA)

Sera diluted in PBS-0.1% Tween-20 (PBS-T) supplemented with 1% skim milk powder (PBS-TM) and 1% BSA (PBS-TMB) were analyzed for reactivity to JKLM ring fragment by indirect ELISA. All incubation steps were performed 1 h at 37°C with 100 µl of reactant unless otherwise stated and separated by washing steps in PBS-T. 96-well microtiter Maxisorp Immunoplates (Nunc) were coated with (JKLM)<sub>10</sub>-OVA conjugate and control antigens in PBS. For antibody kinetics and titration experiments, coating concentrations were set at 5 µg/ml. The following control antigens were examined: i) homologous (BSA) and heterologous (OVA) carrier proteins; ii) previously prepared hapten-OVA conjugates using monensin (Pauillac *et al.*, 1993), PbTx-3 -member of the brevetoxin polyether-type toxins (Naar *et al.*, 1998) and cholesterol (unpublished data). Remaining active sites were blocked by addition of 250 µl of PBS containing 5% skim milk powder. After incubation, bound antibodies were revealed as previously described (Pauillac *et al.*, 1993) using goat anti-mouse IgG antibodies conjugated to β-galactosidase (Biosys, France) and the fluorogenic substrate 4-MUG (4-methyl umbelliferyl-β-D-galactoside). In order to use smaller quantities of reactants, the above procedure was performed in hydrophobic 60-well Terasaki microtiter plates (Labrousse *et al.*, 1982; Pauillac *et al.*, 1993). Briefly, wells were coated with 5 µl of each antigen in PBS (30 ng/ml) then incubation and washing steps were unchanged. This procedure allowed as little as 15 µl of each antibody (primary and secondary) and 20 µl of substrate to be used. Finally bound antibodies were revealed by transferring 15 µl of the wells content into 96-well microtiter plates containing 50 µl of 2N Na<sub>2</sub>CO<sub>3</sub>, and the fluorescence was read as described above.

### Competitive inhibition ELISA

Plates were coated with (JKLM)<sub>10</sub>-OVA solution in PBS either at 1 µg/ml (classical 96-well format) or at 30 ng/ml (60-well Terasaki format). Competitive binding reactions were set up with various levels of inhibitors (JKLM ring fragment, CTX-1B, PbTx-3, nigericin and cholesterol) and fixed antiserum dilutions in PBS-TMB in the range 1/30,000-1/50,000 for the classical format. Using the Terasaki format, the serum dilution was set at 1/14,000. CTX-1B was purified in our laboratory from moray eel liver according to previously published methods (Legrand *et al.*, 1989). Monensin, nigericin and cholesterol were purchased from Sigma (France) and PbTx-3 was from Latoxan (France). Briefly, antigen-antibody (1:1) mixtures were allowed to react directly onto (JKLM)<sub>10</sub>-OVA conjugate-coated plates, then following a washing step, antibodies associated with the plates were revealed as described above. Using the Terasaki format the K<sub>D</sub> value was estimated by calculating the slope of the regression line obtained with the simplified mathematical equation of Klotz (1953) proposed by Friguet *et al.* (1985).

### Simplified extraction procedure of fish tissues

Raw fish fillets of a red snapper (*Lutjanus bohar*) caught in a known ciguatera-endemic area of Tahiti island were homogenized by mixing in a Waring blender for 4 min then divided into 6 samples of 100 g wet weight each for extraction process. Samples were extracted 3 times by MeOH (1/1, w/v), the resulting organic phases were then partitioned between 60% aqueous MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1/2). The methanol layer was pervaporated under vacuum to dryness and the ciguatoxicity of the residue was assessed in one sample using the standard mouse bioassay (Legrand *et al.*, 1989) and expressed as mouse unit equivalents (MU). One MU is defined as the weight of pure toxin or crude toxic extract required to



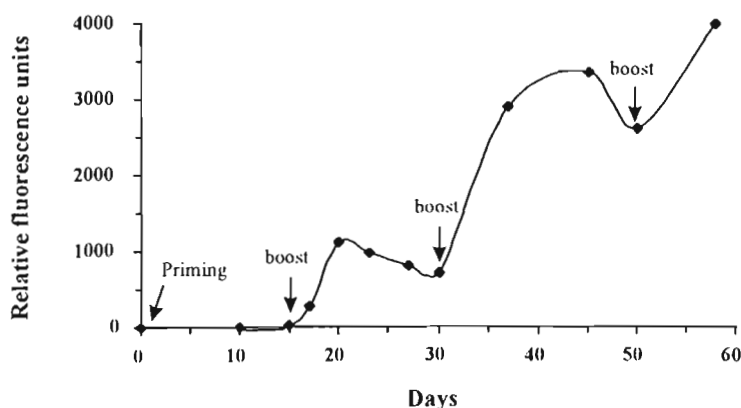


Fig. 2. - Kinetics of anti-JKLM antibodies production.

kill 50% of a batch ( $n = 10$ ) of 20 g mice. For CTX-1B,  $LD_{50} = 0.33 \mu\text{g/kg}$ , hence 1 MU represents 6.6 ng or 6 picomoles. For simplification purpose we assumed that the occurrence of other minor toxins in the extract could be neglected, therefore the whole toxicity ( $0.375 \text{ MU/g}$ ) could be expressed as CTX-1B equivalents ( $(250 \text{ ng/100 g})$ ).

After extraction, the remaining five extracts were dissolved in MeOH (1 ml) and appropriate volumes were dispensed into glass tubes to generate five set of standard in the range of 0.4-22 ng CTX-1B equivalents. The solvent was evaporated under a stream of nitrogen and the residues were solubilized in 100  $\mu\text{l}$  of diluted antiserum (1/30,000) in MeOH/PBS-TM (30/70) then transferred onto 96-well ELISA plate coated with OVA-JKLM conjugate. Antibodies associated with the plates were revealed as previously described.

## RESULTS

### Synthesis and analysis of the conjugates

The conjugation of JKLM ring fragment to BSA and OVA was performed using equimolar ratio of hapten/lysine residues (Fig. 1). The analysis of the purified conjugates by trinitrophenylation (Habeeb, 1966) gave an epitope density of 16 hapten molecules per molecule of BSA and 10 per molecule of OVA, indicating that the overall conjugation efficiency was  $\approx 25\%$ .

### Production of anti-JKLM antibody

In a preliminary experiment, mice injected with both BSA and OVA conjugates displayed no ill-effects or symptoms typical of ciguatera. Later, the BSA and OVA conjugates were used as immunogen and testing antigen, respectively. All preimmune sera were totally negative against a panel of coating antigens, including the homologous and heterologous native carrier proteins (data not shown). That mice developed specific immune response upon immunization was clearly demonstrated by the antibodies kinetics after each injection (Fig. 2), with only slight differences observed between the two antisera. Mice produced a moderate but significant antibody titers as soon as 7 weeks after initial injection. Serum titration of the best responder after the 4th injection is reported in figure 3. As can be seen, serum dilutions exhibit JKLM specificity in an indirect classical ELISA

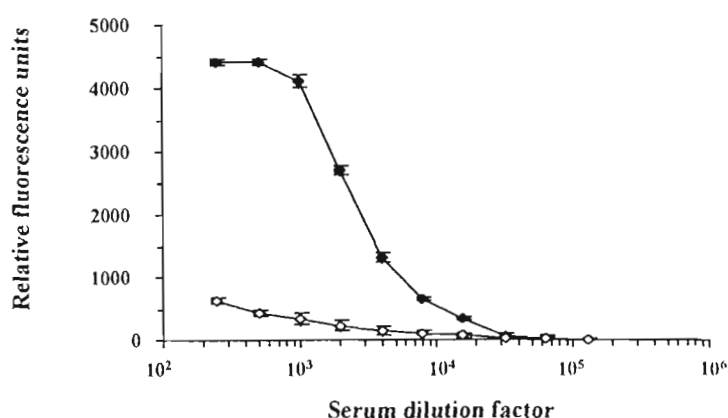


Fig. 3. - Titration of mouse anti-JKLM serum. (JKLM)<sub>10</sub>-OVA coating solutions were set at 5 µg/ml PBS and 2-fold serial serum dilutions made in PBS-TM. Each value represents the mean of triplicate assays with error bars indicating  $\pm$  SD.

format onto (JKLM)<sub>10</sub>-OVA-coated plates and lower antibody concentrations yield signals similar to background level (control without antibody). Moreover it was later observed that cross-absorbing the anti-JKLM-BSA sera with 100 µg/ml of BSA completely neutralized the anti-carrier activity in the serum (see below). Therefore titers estimated from the maximum serum dilution that allowed a 3-fold signal to testing antigen compared to background level were  $\approx$  1/16,000 when coating antigen concentration was set at 5 µg/ml.

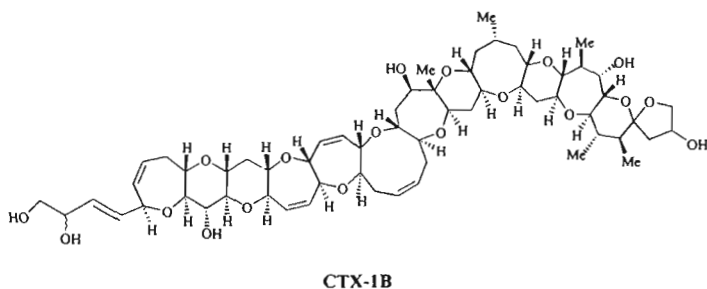
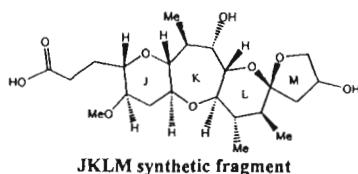
#### Specificity of anti-JKLM antibody using the classical ELISA format

The specificity of anti-JKLM antibody was first demonstrated by indirect ELISA using various coating antigens at fixed protein concentrations (1 µg/ml) and a unique pooled normal ( $n = 10$ ) or immune ( $n = 2$ ) serum dilution (1/50,000) made either in PBS-TM or PBS-TMB. As is shown in table I, the addition of BSA in the dilution buffer completely inhibited the anti-carrier antibodies at the level of the background obtained with the pooled normal serum sample. Hence true differential antibody reactivity to the haptens could be easily deduced from these experiments. Compared to high binding activity to (JKLM)<sub>10</sub>-OVA, only slight reactivity to other haptens, including the polyether-

Table I. - Specificity of mice polyclonal antibodies by indirect ELISA. 96-well standard plates were coated with the following antigens solutions made 1 µg/ml in PBS: OVA, BSA, OVA-Chol, OVA-Mon, OVA-PbTx-3 and OVA-JKLM. Pooled normal ( $n = 10$ ) or immune ( $n = 2$ ) sera were diluted (1/50,000) either in PBS-TM or PBS-TMB. Values are expressed as mean relative fluorescence units ( $n = 5$ ). The respective coefficients of variation were found between 3-7% (data not shown).

ELISA format	Serum dilution factor	Detection limit (picomole)	
		JKLM	CTX-1B
Classical	30,000	50	30
	50,000	ND	7
Terasaki	14,000	0.6	ND

A



B

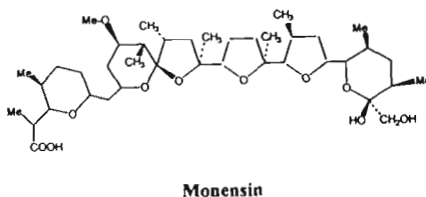
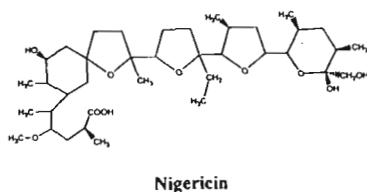
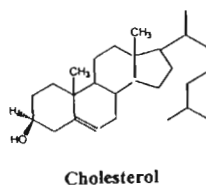
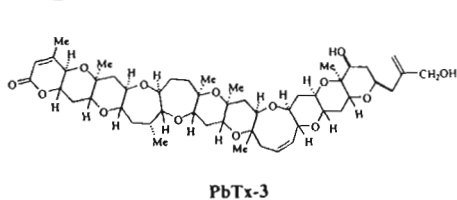


Fig. 4. - Chemical structures of the haptens tested for antibody specificity. **A:** Structures of the JKLM synthetic ring fragment and CTX-1B. **B:** Structure of other polyether or steroid compounds.

type PbTx-3 was observed. The excellent reproducibility of this ELISA was evident from the calculation of the coefficients of variation ( $n = 5$ ) which ranged between 3 and 7%.

In order to further assess the specificity of the anti-JKLM antibodies, competitive ELISA experiments were performed using various inhibitors (Fig. 4A, B). They were chosen among polyether toxins with related structures (CTX-1B and PbTx-3) or with antibiotic properties (nigericin and monensin). Cholesterol, a non-related steroid structure, was tested to ascertain the polyether selectivity of the assay. The high cross-reactivity of the anti-JKLM antibodies to CTX-1B was again clearly demonstrated, since all the other free haptens tested were unrecognized by the antibody (data not shown). Standard curves were obtained by plotting percent binding relative to control experiment

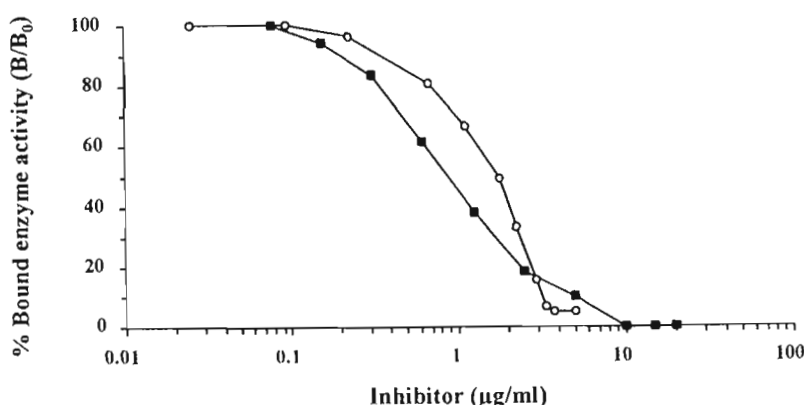


Fig. 5. - Classical ELISA format: calibration curves for JKLM fragment and pure CTX-1B. Competitive inhibition of PABs binding to (JKLM)<sub>10</sub>-OVA conjugate-coated plates by free JKLM (■) or CTX-1B (○). Antigen coating solutions were set at 1 μg/ml PBS and the final serum dilution was 1/30,000 in PBS-TMB. Each point represents the mean of 3 independent determinations. Error bars are omitted because of symbol overlapping.

(B/B<sub>0</sub>) versus JKLM fragment and CTX-1B concentrations (Fig. 5). It was observed that CTX-1B exhibited a similar inhibitor activity ( $IC_{50} = 1.67 \mu\text{g/ml}$  or  $1.5 \times 10^{-6} \text{ M}$ ) as the synthetic ring fragment used for immunization ( $IC_{50} = 0.89 \mu\text{g/ml}$  or  $2 \times 10^{-6} \text{ M}$ ). With this fragment as inhibitor and assuming a difference of 3 standard deviations ( $n = 10$ ) from the mean signal without inhibitor is significant, the detection limit of the assay was  $0.22 \mu\text{g/ml}$  ( $5 \times 10^{-7} \text{ M}$ ) for JKLM ring fragment and  $0.33 \mu\text{g/ml}$  ( $3 \times 10^{-7} \text{ M}$ ) for CTX-1B. Since the total volume deposited onto microtiter wells equals 100 μl, it appears that 22.2 ng (50 picomoles) JKLM ring fragment and 33.3 ng (30 picomoles) CTX-1B are the smallest quantities clearly detectable in the assay.

#### Competitive microELISA standard curve for JKLM ring fragment

Figure 6 shows a JKLM calibration curve obtained with the previously reported competitive microELISA format performed in Terasaki plates. The detection limit of the assay was 17.8 ng/ml ( $4 \times 10^{-8} \text{ M}$ ) and considering the total volume deposited onto microtiter wells equals 15 μl, it appears that 0.27 ng (0.6 picomole) JKLM ring fragment is the smallest quantity clearly detectable. In this assay the  $K_D$  for the interaction of free JKLM fragment and the antiserum was found to be  $2 \times 10^{-7} \text{ M}$ .

#### Evaluation of the competitive classical ELISA format for detection of CTXs in fish tissue

For further evaluation of the ability of mice PABs to react with CTX-1B or related congeners, a simplified extraction procedure was used to assess CTXs in crude lipid extracts of known toxicity (37.5 MU/100 g or 250 ng CTX-1B equivalents/100 g) using the classical ELISA format. Five separate extracts from the same highly toxic specimen of red snapper were used as described in Materials and Methods to generate five sets of standards in the range of 0.4-22 ng CTX-1B equivalents were mixed with diluted antiserum (final concentration range:  $4 \times 10^{-9}$  -  $2 \times 10^{-7} \text{ M}$  CTX-1B). A typical standard curve is presented in figure 7 where each point was tested in triplicate. The interassay coefficient of variations ( $n = 5$ ) at various concentrations varied between 3-9% with the higher value

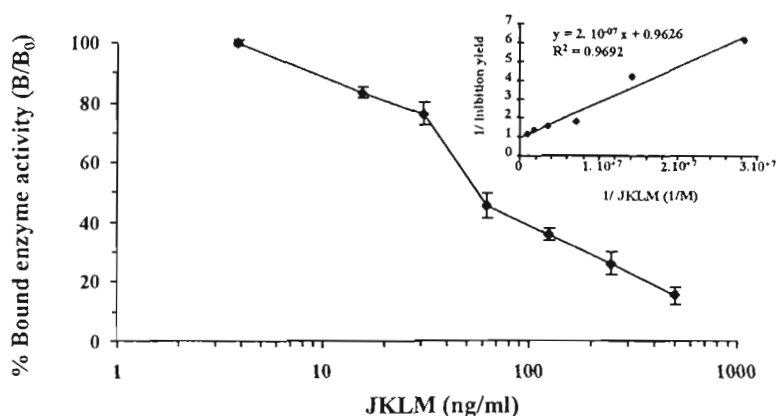


Fig. 6. - Terasaki ELISA format: calibration curve for JKLM fragment. (JKLM)<sub>10</sub>-OVA coating solution was set at 30 ng/ml PBS and the final serum dilution was 1/14,000 in PBS-TMB. Each point represents the mean of 3 independent determinations with error bars indicating  $\pm$  SD.

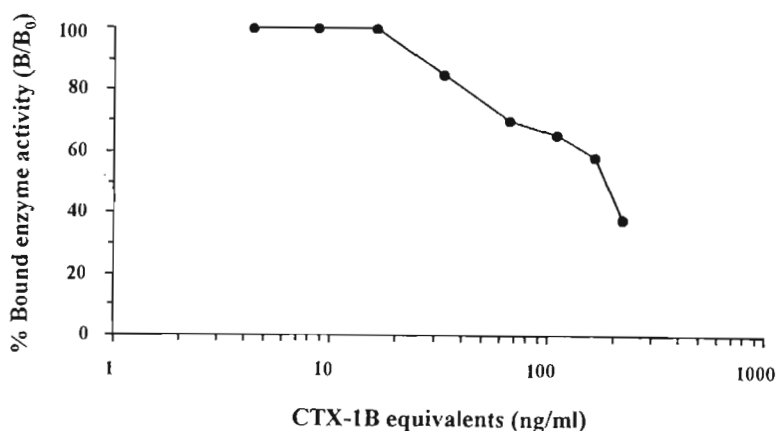


Fig. 7. - Assessment of CTXs in crude lipid extract of carnivorous fish (*Lutjanus bohar*). (JKLM)<sub>10</sub>-OVA coating solution was set at 1  $\mu$ g/ml PBS and the final serum dilution was 1/50,000 in PBS-TMB. Each point represents the mean of triplicate determinations. Error bars are omitted because of symbol overlapping.

corresponding to the greater concentrations. The same calculation as above led to a limit of detection of  $\approx$  8 ng of CTX-1B equivalents per well (7 picomoles).

#### Comparison between the two ELISA formats

Table II summarizes the performances of the two immunoassays and give some insight into the applicability of Terasaki plates to help CTXs detection in rapid flesh extracts. On the one hand, using the classical format, a slight decrease (1.67-fold) in antibody concentration results in a 4-fold decrease of CTX-1B detection limit (from 30 to 7 picomoles). On the other hand, regarding JKLM, the use of smaller reactant volumes in Terasaki plates necessitates a 2-fold increase of antibody concentration but yields a 80-

fold lower detection limit than the one allowed by classical format (0.6 picomoles instead of 50).

## DISCUSSION

Besides CTXs, *Gambierdiscus toxicus* as either wild or cultured specimen produces significant amount of water-soluble polyether type toxins named maitotoxins (MTXs). The three MTXs so far reported (Holmes and Lewis, 1994) are highly potent, slow acting toxins specific for the calcium channel but have no proven role in causing human illness associated with ciguatera. Hence the challenge to ensure public health is to develop an effective screen for CTXs containing fish. Due to the lack of specificity and sensitivity of the previously reported bioassays and despite the development of very sensitive but highly sophisticated detection methods (Legrand *et al.*, 1992; Pauillac *et al.*, 1995; Lewis *et al.*, 1999), the immunological approach appears more valuable to routinely assess CTXs in fish tissue. Although previous studies have emphasized the multiplicity of fish CTXs (Murata *et al.*, 1989; Lewis and Sellin, 1992; Satake *et al.*, 1993, 1997; Legrand *et al.*, 1989, 1992, 1998), it is not until recently that precise toxin profiles of carnivorous and herbivorous pacific fish have been correctly addressed, with the main targets being CTX-1B (i.p. LD<sub>50</sub> into mice = 0.33 µg/kg) and CTX-2B1 (LD<sub>50</sub> = 0.8 µg/kg) which contribute ≈ 80% of total carnivorous fish toxicity, and CTX-3C (LD<sub>50</sub> = 2.5 µg/kg) which contributes at least 60% of total herbivorous fish toxicity (Legrand *et al.*, 1997).

In this context, we have produced the first mice Pabs specific to CTX-1B, the more potent and more representative CTX extracted from carnivorous fish, using a synthetic tetracyclic JKLM fragment of CTX-1B. A three-carbon carboxylic spacer-arm attached to the first ring of this synthetic CTX motif was used to covalently link the hapten to carrier proteins leading to conjugates with epitope density within the optimal range to ensure hapten-specific antibody production (Erlanger, 1980). This immunogen construction was chosen to enable a good presentation of the right-hand terminus of CTX-1B because it has been previously demonstrated that increasing the length of the chemical spacer can severely affect the production of hapten-specific antibodies (Beckett *et al.*, 1978); and as an artifact, it can also be part of the epitope recognized by the induced antibodies (Weyrer *et al.*, 1990). Finally, the relevance of our construction is emphasized by recent data on the

Table II. - Comparative immunoassay performances. Pooled immune serum (n = 2) samples diluted in PBS-TMB and mixed with various amounts of competitors (JKLM fragment or CTX-1B) were allowed to react directly onto wells of classical or Terasaki plates precoated with (JKLM)<sub>10</sub>-OVA solution (see Materials and Methods). Detection limits expressed as picomoles hapten were calculated as described in the text.

Antigen*	Serum**		
	NS (PBS-TM)	IS (PBS-TM)	IS (PBS-TMB)
OVA	30	316	48
BSA	40	3665	61
OVA-Chol	34	267	40
OVA-Mon	50	298	52
OVA-PbTx-3	36	342	42
OVA-JKLM	42	2500	2098

chemical structure of other CTX congeners (Murata *et al.*, 1989; Legrand *et al.* 1989, 1992, 1998; Lewis and Sellin, 1992; Satake *et al.* 1993, 1997) together with the toxin profile of carnivores and herbivores (Legrand *et al.*, 1997) which have revealed the presence of the JKLM motif in other relevant toxins (CTX-2B1 and CTX-3C).

Successful production of anti-JKLM antibody allowed us to develop two highly specific competitive ELISA formats. Some earlier studies using either sheep PABs to CTX-1B (Kimura *et al.*, 1982; Hokama *et al.*, 1983, 1984; Hokama, 1985) or Mabs to CTX-1B or OA (Hokama *et al.*, 1985, 1988, 1992) or goat PABs to PbTx (Baden *et al.*, 1985) did claim the existence of shared epitopes among polyether compounds, including MTXs in some studies. It is noteworthy that Poli and Heweston (1992) using another goat antiserum raised against PbTx-3 were unable to detect any cross-reactivity to other polyether-type toxins including palytoxin and OA and its derivatives. In this study, when free haptens other than JKLM ring fragment or CTX-1B were used as competing analytes in the classical format, our mice PABs showed no cross-reactivity indicating their high specificity towards the JKLM motif. Moreover on a molar basis, PABs exhibited a higher cross-reactivity (133%) against CTX-1B itself indicating a preferential reactivity with the natural epitope. This result accounts for the lower limit of detection obtained with CTX-1B (30 picomoles) compared to JKLM (50 picomoles).

Although preliminary titration experiment revealed a 1/16,000 antiserum titer, it is noteworthy that in competitive binding experiments using a 1/30,000 or 1/50,000 serum dilution, reduction of the coating concentration from 5 to 1 µg/ml was effective in increasing the sensitivity of the assay (Table II). This result is in good agreement with earlier observation (Nieto *et al.*, 1984) that decreasing the coating antigen concentration allowed different antibody subpopulations with increased affinities to be selected. The same explanation would stand for the Terasaki format where coating concentration was reduced to 30 ng/ml but the limitation arose from Bo value (signal without inhibitor), hence a good compromise was to use a 1/14,000 antiserum dilution (15 µl). Nevertheless, because of the limited number of free antibody sites of higher affinity, as little as 17.8 ng/ml (0.6 picomole/well) JKLM ring fragment could be detected in this binding inhibition assay.

Anti-JKLM antibodies were found to react with CTXs containing crude lipid extracts with high affinity, and apparently no clear matrix effects were observed since the same detection limit was attained as with pure CTX-1B in PBS-TM (7 picomoles/well in the classical ELISA format).

Usually the matrix effects involve interactions of the matrix, the analyte, and the technology base of the test method. Regarding the matrix itself, our system benefited from optimum conditions due to the fact that 1) carnivorous fish generally contain less non-toxic lipid-soluble contaminant substances than herbivorous ones and 2) the *Lutjanus bohar* specimen used was found to be highly toxic. Regarding the analyte, presence in the extract of other less toxic cross-reacting CTXs congeners - that have not been taken into account in the CTX-1B equivalent conversion system - might cause positive interferences since spiking non-toxic extract with pure CTX-1B did reveal a slight matrix-effect (unpubl. data). We postulate that in our study these facts taken together could have contributed to counterbalance the well-known matrix effects. Hence, experiments where a known amount of toxin is tested in the presence of increasing amounts of matrix from different origin (carnivorous and herbivorous) could confirm these effects.

The principal requirements for CTXs immunodetection are sensitivity and specificity. As a first step in the search for greater sensitivity, a competitive microELISA format

for JKLM ring fragment was developed in Terasaki plates with detection limit around 0.27 ng (0.6 picomoles). Taking into account the high PABs cross-reactivity to CTX-1B and assuming that as low CTXs levels as 0.1 ng/g of carnivorous fish flesh are to be detected, a 80% extraction efficiency combined to a 100-fold concentration factor provided by an additional clean-up procedure would appear to be sufficient to evaluate ciguatera toxicity in a 10 g flesh sample (0.8 picomoles CTX-1B equivalent).

Due to the lack of Terasaki plates reader, relative fluorescence associated with each well was measured by transfer into 96-well plates. Assuming that this step is time-consuming and laborious, in order to increase the sensitivity and specificity of this antibody-based assay in 96-well plates, a number of approaches will be further investigated according to the experimental design techniques proposed by Sittampalam *et al.* (1996) but using other signal amplification methods. This methodology will enable a thorough study of factors that minimise any non-selective binding of CTXs to IgG and the non-selective binding of IgG to fish tissue (Berger and Berger, 1979; Laigret *et al.*, 1981; Chanteau *et al.*, 1981; Emerson *et al.*, 1983).

These precautionary measures constitute an absolute prerequisite to avoid the high rate of false negative and false positive previously reported in a study of Caribbean fish (Dickey *et al.*, 1994) with the Ciguatetect™ kit of Park *et al.* (1992) using MABs from Hokama's laboratory. Finally this assay must be checked for reproducibility and accuracy compared with the mouse bioassay or other analytical (HPLC) and detection assays (receptor binding and cell-based assays), using toxic and non-toxic specimens of the same species. MABs production to JKLM ring fragment is underway in order to facilitate the routine assessment of CTXs in fish tissue at the laboratory level.

On the assumption that the challenge is to detect a family of closely related structures, cross-reaction with other structurally and toxicologically characterized CTXs will be investigated, but it is clear that the JKLM motif cannot always exist. Hence to help the detection of non-JKLM cross-reacting CTXs congeners, we have developed a strategy for preparing specific antibodies using small amount of hapten. As molecular model we used only 400 µg of PbTx-3 that could be successfully linked to BSA and OVA (Naar *et al.*, 1998). Following mice immunization with (PbTx-3)<sub>18</sub>-BSA conjugate, highly specific antibodies have been produced with  $K_D$  values in the range of  $10^{-8}$  M enabling detection limits of 0.33 picomoles hapten in ELISA or rapid RIA formats. Although no cross-reactivity with CTX-1B was observed, these results confirmed the potential in preparing immunogen with very rare haptens whose low detection level still remains problematic.

On the basis of our results, it is postulated that in the future a sandwich-type assay could be developed from MABs obtained from the JKLM and the ABC rings of CTX-1B or its congeners. With such antibodies it should be possible to develop low-cost, rapid, simple, specific and sensitive kits for personal use either at home or in the field, or for commercial use at various points prior to sale.

**Acknowledgments.** - This work was supported by grants from the Government of French Polynesia (Tahiti), Pasteur Institute (Paris, France) and CORDET Commission (Paris, France).



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## **LABORATORY DIAGNOSTICS OF BREVETOXIN AND CIGUATOXIN INTOXICATIONS IN HUMANS: CASE REPORTS AND SAMPLING CONSIDERATIONS**

by

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**ABSTRACT.** - Marine biointoxications can be a source of difficulty and intrigue for many clinicians. Diagnosis can be difficult due to their relative rarity and symptoms which can appear nonspecific if a history of seafood ingestion is not provided to the medical caregiver. Laboratory evaluation of ingested foodstuffs or clinical samples can provide critical diagnostic information, but only if these samples are collected promptly and handled appropriately. In addition, clinical samples can provide insight into the underlying pharmacokinetics of the intoxication as well as help improve and validate the assays. This paper describes two recent clinical outbreaks: an outbreak of ciguatera poisoning in Haiti and an outbreak of neurotoxic shellfish poisoning in Florida. In each case we discuss the samples collected and the information gathered from those samples. Finally, we discuss how improved sampling might have provided additional information and give recommendations for sample collection during future outbreaks.

**RÉSUMÉ.** - Analyses de laboratoire pour diagnostiquer les intoxications humaines par les brévétoxines et ciguatoxines: exposés de cas et conditions des prélèvements.

Les biointoxications marines peuvent être une source de difficultés et d'intrigues pour beaucoup de cliniciens. Le diagnostic peut être difficile en raison de leur relative rareté et des symptômes qui peuvent apparaître comme étant non spécifiques si un historique de l'ingestion marine n'est pas fourni au personnel médical. L'évaluation en laboratoire des produits alimentaires ingérés ou des échantillons cliniques peut apporter des informations critiques pour le diagnostic, mais seulement si ces échantillons sont recueillis immédiatement et traités de façon appropriée. De plus, les échantillons cliniques peuvent fournir un aperçu des pharmacocinétiques à l'origine de l'intoxication ainsi qu'une aide pour améliorer et valider les dosages. Ce papier décrit deux manifestations cliniques récentes: une manifestation d'empoisonnement ciguatérique à Haïti et une manifestation d'empoisonnement par des fruits de mer neurotoxiques en Floride. Dans chaque cas, nous discutons les échantillons recueillis et les informations accumulées à partir de ces échantillons. Finalement, nous discutons comment le prélèvement amélioré d'échantillons aurait pu fournir des informations supplémentaires et nous donnons des recommandations pour la collecte des échantillons à l'occasion des prochaines manifestations de la maladie.

**Key-words.** - Ciguatoxin, Brevetoxin, Clinical outbreaks, Diagnostics.

Diagnosis of marine toxin poisoning can present difficult problems to the medical and scientific communities. These events are relatively uncommon, and symptoms can appear nonspecific if the history of seafood ingestion is not provided to the medical

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caregiver. Even if this information is given, mild to moderate intoxication can be difficult to diagnose for the inexperienced. For this reason, many cases of intoxications go undiagnosed or diagnosis is delayed for several days. Laboratory evaluation of foodstuffs or clinical samples can provide critical diagnostic information, but only if these samples are collected promptly and handled properly. Once samples are collected and transported to the laboratory, other potential problems arise: sample sizes may be small, limiting diagnostic options; often the pharmacokinetics or metabolism of the compound in question are not well known; stability may be an issue. For these reasons, it is often better to evaluate a sample of the ingested foodstuff rather than clinical samples. However, sometimes only one or the other is available. In the best-case scenario, both are available and multiple clinical samples are collected during the relevant time frame. This provides the maximum information to both the clinician and the scientist and can result in significant future assay improvement as well as a better understanding of the pharmacological events underlying the clinical syndrome.

Sensitive assays now exist for brevetoxins and ciguatoxins in biological matrices. Although not yet clinically validated for hospital use, they remain valuable tools and excellent diagnostic aids in the research laboratory. In this paper we shall describe two separate outbreaks of seafood poisoning and how the collection of samples directly affected the information which was ultimately garnered by the scientific community and provided back to the medical caregiver.

## CASE REPORTS

### Case #1: An outbreak of ciguatera fish poisoning in Haiti

On 24 February 1995, six U.S. soldiers serving with the Multinational Force (MNF) in Haiti became ill after attending a beach barbecue (this outbreak is described in detail in Poli *et al.*, 1997). The main course at this meal was a large amberjack (*Seriola dumerili*) caught approximately 80 km from Port-au-Prince by a local fisherman. Within 1-6 h after the meal, the soldiers developed symptoms characteristic of ciguatera fish poisoning. All were seen at the MNF battalion aid station 5-8 h after the meal with gastrointestinal, cardiovascular and neurological complaints. Three patients developed bradycardia and hypotension and one was admitted to intensive care with systolic blood pressures of 60-70 mm Hg. Gastrointestinal and cardiovascular symptoms abated in all patients within 72 h. However, four patients still complained of pruritus and paresthesias of the extremities 3 weeks after discharge. All patients recovered fully in 1-3 months. All six victims were seen by a single physician who made the presumptive diagnosis of ciguatera poisoning based upon symptomatology and the history of ingestion of fish. No urine or blood samples were collected at the time of the presumptive diagnosis.

Immediately after the dinner, one soldier returned to his barracks with a portion of fish to share with a friend who was unable to attend the party. Upon diagnosis of ciguatera, this piece of fish was recovered and transported to Fort Detrick for analysis. No attempt was made to procure any fish that may have remained from the party.

The piece of cooked fish (326 g cooked weight) was analyzed for the presence of ciguatoxins by a receptor binding assay. This assay is described in detail elsewhere (Poli, 1996; Poli *et al.*, 1997; Trainer and Poli, 1997). Briefly, fish tissue must first be extracted to remove the toxic materials. This was done by homogenization in acetone followed by filtration and evaporation to dryness. The residue was then dissolved in aqueous methanol

and extracted with hexane to remove neutral lipids. The methanol fraction was then partitioned into chloroform and the methanolic fraction discarded. The chloroform fraction was applied to a silica gel column and unbound material washed away. Bound material was eluted with 10% methanol in chloroform. This semipurified ciguatoxic fraction was then tested in the membrane assay.

The membrane assay is based upon the knowledge that ciguatoxins exert their toxic effects by binding to a specific receptor site on the voltage-sensitive sodium channels in mammalian nerve cells. This specific receptor site also binds brevetoxins, the neurotoxic shellfish toxins produced by the Florida red tide dinoflagellate *Gymnodinium breve* (Poli *et al.*, 1986). In this assay, samples are analyzed for their ability to specifically compete with radiolabeled brevetoxin for receptor sites on rat brain membranes. Comparison to a standard curve allows quantitation of activity; detection limits are approximately 0.1 ng brevetoxin or ciguatoxin. Analysis of the Haiti sample demonstrated that this fish contained ciguatoxin activity equivalent to approximately 20 ng ciguatoxin/g fish tissue, one of the most toxic samples ever seen in this laboratory. The presence of ciguatoxin was confirmed by liquid chromatography/mass spectroscopy, which detected the Caribbean ciguatoxin parent ion mass as well as the characteristic fragment ions in the extract. This information was immediately relayed back to the MNF aid station physician to confirm his very astute presumptive diagnosis.

In this case, we were able to confirm the presumptive diagnosis of ciguatera fish poisoning within a matter of days. However, by the time this information reached the physician it was too late to collect any remaining toxic fish. More importantly, it was also too late for any evidence of ciguatoxin activity to be detected in urine or serum samples. Little is currently known about either the pharmacokinetics/excretion or the metabolism of ciguatoxins in humans. Had urine and serum samples been taken immediately and either held under the proper storage conditions or sent with the fish sample, important scientific data may not have been lost.

#### **Case #2: An outbreak of neurotoxic shellfish poisoning (NSP) in Florida**

On 15 June 1996, three family members became ill after ingesting shellfish taken from Sarasota Bay, Florida. The family members consisted of an adult male, his 2-year-old son and his 3-year-old daughter. Each had ingested whelks (*Busycon contrarium*), which had been boiled for 10-20 min. The amount ingested for each patient was not available. Approximately one hour after eating, the father developed perioral numbness, numbness in the extremities, and vomiting. The children were awakened from sleep and immediately complained of abdominal pain and began to vomit. All three victims were seen in the local emergency room approximately 3 h post-ingestion. The father was stable upon admittance and complained only of nausea and fatigue. He was discharged after 4 h. The male child lost consciousness and experienced convulsions on the way to the hospital. The female child was actively vomiting, tachycardic (pulse 130), complained of shortness of breath upon admittance and experienced grand mal seizures approximately 30 min later. Both children were intubated and stabilized, and both were discharged from the hospital after 2 (male) or 3 (female) days. Urine and serum specimens were collected from both children upon initial examination and during a followup visit to their pediatrician 4 days later.

The shellfish were supplied to the family by a friend who routinely harvested seafood from the bay for his own consumption. Interviews with the family and the supplier

resulted in the location of harvest and an empty shell from the night of the meal. This empty shell was the basis for the identification of the offending organism. Approximately 1 month after the incident, additional whelks were harvested from the same location, frozen, and shipped to the U.S. Food and Drug Administration for analysis.

Upon receipt of samples, the whelks were thawed, removed from their shells and separated into digestive tissue and feet. Each sample was homogenized in acetone and filtered. The acetone fraction was evaporated to dryness and redissolved in aqueous methanol. This methanolic fraction was then extracted with hexane to remove neutral lipids. The hexane fraction was discarded and the methanolic fraction evaporated to dryness and redissolved in fresh methanol. These crude extracts were then analyzed for brevetoxin activity by the membrane assay described above and also by a radioimmunoassay specific for the PbTx-2-type brevetoxins (Poli and Hewetson, 1992). The results are shown in table I. The extracts of the digestive glands contained a large amount of brevetoxin activity in the radioimmunoassay (11.2 and 22.0  $\mu\text{g/g}$ ), while feet contained much lower activity (2.5  $\mu\text{g/g}$ ). The membrane assay gave qualitatively similar results, although the calculated numbers were somewhat lower. Both assays indicated that whelks contained brevetoxin in excess of the regulatory limit (0.8  $\mu\text{g/g}$ ), which confirmed toxic activity in the same species of shellfish ingested by the victims and harvested in the same location.

Although this information certainly suggests that brevetoxins caused the illness to this family, we were unable to analyze the actual ingested material and therefore had no concrete connection between the toxic shellfish and the victims. Luckily, the emergency room physicians had the foresight to collect urine and serum samples upon admittance, and later samples were taken during a followup examination.

Pharmacokinetics experiments performed previously in rats (Poli *et al.*, 1990) demonstrated that brevetoxin (PbTx-3) is excreted fairly rapidly. The majority of the administered toxin was eliminated in the first 48 h and elimination was virtually complete after 3 days. Although the main route of elimination was via the feces, a significant portion (approx 14%) was eliminated in the urine. This makes urine a viable option in terms of testing for previous exposure, as long as the samples are collected within the first 48-72 h. In the case of the Florida children, we were particularly fortunate: urine was collected within hours of intoxication, and again 4 days later. This meant that we not only had a sample to test, but each assay could be performed in parallel with an internal control sample to control for possible matrix effects. The results are shown in table II. Both children had high levels of brevetoxin activity in their urine at the time of admittance to the emergency room, but there was no trace of toxin 4 days later when they were fully recovered. This data allowed us to confirm the diagnosis of neurotoxic shellfish poisoning in this outbreak.

Table I. - Analysis of whelks (*Busycon contrarium*) collected at the location of those implicated in an outbreak of neurotoxic shellfish poisoning. Whelks were separated into digestive tissue and feet and pooled as indicated. Lipid extracts were prepared as described in the text and the extracts tested for brevetoxin activity by specific radioimmunoassay and membrane binding assay. Results are expressed as the mean ( $\pm$  SD). The regulatory limit for brevetoxins in Florida is 0.8 mg/g.

	Radioimmunoassay ( $\mu\text{g/g}$ ) (n = 3)	Membrane assay ( $\mu\text{g/g}$ ) (n = 3)
Digestive (1)	22.0 ( $\pm$ 5.0)	9.2 ( $\pm$ 3.6)
Digestive (2 pooled)	11.2 ( $\pm$ 1.6)	6.5 ( $\pm$ 1.6)
Feet (3 pooled)	2.5 ( $\pm$ 0.5)	0.6 ( $\pm$ 0.1)



## DISCUSSION

Both neurotoxic shellfish poisoning and ciguatera poisoning are difficult problems from a scientific or medical standpoint. Although the causative agents are known in each case and the clinical manifestations are well-described, confirmation of exposure is still problematic. Diagnoses are based upon symptomatology and history of eating the appropriate seafood. Although laboratory tests are available to detect the parent toxins, virtually no information exists regarding the toxicokinetics, distribution, or elimination of these compounds in humans. These data can only be gathered from analysis of samples collected during outbreaks of seafood poisoning. For this reason, it is critical that proper samples be collected at the time of diagnosis and immediately thereafter.

In the outbreak of ciguatera described above, we were fortunate to obtain a sample of the offending fish for analysis. Without the thoughtfulness of one soldier in taking a portion of fish for his friend, we could not have confirmed this diagnosis. It is always important to try to obtain as much of the tainted food as possible. In addition to aiding in diagnostics, this material can be important for a better understanding of the basis of the clinical syndrome. In this case, from the small piece of fish collected from the soldier, we were able to identify two previously unknown minor ciguatoxins (Poli *et al.*, 1997). Had a concerted effort been made at the time of the presumptive diagnosis to collect all remaining material from the meal, perhaps we might have had sufficient material to have found one or more of the other minor Caribbean ciguatoxins that have since been described (Vernoux and Lewis, 1997).

In the case of the NSP outbreak in Florida, it was possible to collect additional toxic material from the same location. Analysis of this material demonstrated that the toxins ingested by humans differed significantly from those produced by the dinoflagellate. Toxicity in the whelk tissues consisted of several fractions which differed from the parent toxins (unpubl. data). These fractions are currently under investigation, and may have important implications for regulatory testing.

The physicians who examined the patients in Florida had the foresight to take blood and urine samples upon admittance. From these urine samples, we were able to confirm exposure to brevetoxins, the first time this has been accomplished in NSP victims. The urine samples taken at the followup visit 4 days later were free of brevetoxins, thus correlating the assay results with the resolution of symptoms and giving us an outer limit of elimination time that agreed well with animal data. However, had additional urine collections been made at interim time points, we might have gathered important pharmacokinetics data relating to elimination of brevetoxins in humans. Had larger volumes of urine been collected, it might have been possible to analyze for the presence of possible brevetoxin metabolites. This kind of information is critical when designing or optimizing appropriate testing methods.

Table II. - Analysis of urine from two victims of neurotoxic shellfish poisoning in Florida. Urine samples were diluted in saline (1:10-1:500) and tested in a specific radioimmunoassay for brevetoxin activity. Day 1 urine was collected within 3 h of intoxication. Day 4 urine was collected at a followup exam after complete recovery from symptoms. A membrane binding assay was not performed due to insufficient sample.

Patient	Day 1	Day 4
Male, age 2 years	83 ng/ml	0
Female, age 3 years	235 ng/ml	0

Had urine been collected from the soldiers in Haiti, would we have been able to detect ciguatoxin or ciguatoxin metabolites? Perhaps. Chemically, ciguatoxins possess a cyclic polyether skeleton very similar to that of the brevetoxins. They have similar lipid solubilities, they can be extracted from tissues by using the same techniques, and they possess the same mechanism of action. Finally, they can be detected by the same membrane binding assay used to detect the brevetoxins. Would they be excreted in the urine? We do not know. However, it is likely that these compounds are metabolized as well. If so, they would likely have appreciable water solubility and thus be excreted in the urine. However, the potency and pharmacokinetics of these two classes of toxins are vastly different. The ciguatoxins are 2-3 orders of magnitude more potent than brevetoxins in the mouse bioassay, suggesting that a much lower ingested dose may be needed to evoke symptoms of intoxication. In addition, as demonstrated in the outbreaks described above, the time course of the two illnesses differ greatly. Neurotoxic shellfish poisoning typically resolves in 48-72 h, while ciguatera poisoning often persists for weeks or months. Thus, even if the toxins (or metabolites) demonstrate appreciable urinary elimination, the size of the ingested dose may be sufficiently small and/or the rate of elimination may be sufficiently slow that the concentration in urine may be below the detection limit of the assay. The answers to these questions will determine whether confirmatory diagnostic assays can be developed for ciguatera poisoning, and these questions can only be addressed through collection of appropriate samples from clinical cases.

Although analysis of serum samples did not play a major role in either outbreak, the importance of collection should not be underestimated. Animal studies currently suggest that brevetoxin levels circulating in the bloodstream are likely below the detection limits of current assays. In the Florida outbreak, insufficient serum was available to adequately address this question. No information is available for ciguatoxins, but because of their chemical similarity to brevetoxins and extremely high potency, we believe that they would also go undetected in serum by current technologies. Potential metabolites, however, may demonstrate longer serum half-lives; this question can only be answered through the analysis of clinical samples. An opportunity to collect these samples should not be missed. As detection technologies advance, validation will rely on clinical samples. Because outbreaks of these kinds are relatively rare, a library of documented archived serum samples complete with patient and outbreak information would be a valuable asset. Physicians should keep this in mind when evaluating patients suspected of suffering from exposure to any marine biotoxin.

## CONCLUSIONS AND RECOMMENDATIONS

Laboratory diagnostic assays can be a great help in the delineation of marine biotoxin poisonings in a clinical setting. By the same token, clinical outbreaks are crucial not only to the continued refinement of diagnostic assays, but to a better understanding of the biochemistry and pharmacology of these compounds in the human body. To that end, it is very important that the proper samples and information be collected whenever a marine biointoxication is suspected. For the maximum benefit to both the scientific and medical communities, we recommend the following:

1. Collect as much urine as possible over the first 72 h. If possible, collect the total volume of each urination. Keep each sample separate and label as to date and time. Freeze if possible, otherwise refrigerate. Slight acidification (to pH 5-6) with acetic acid

and addition of a bacteriostatic agent such as thimerosal or sodium azide may help to preserve refrigerated samples.

2. Collect serum samples every 12-24 h. Separate cells as soon as possible to minimize hemolysis, which interferes with current diagnostic assays. Freeze or refrigerate as soon as possible.

3. Every attempt should be made to recover as much of the ingested material as possible. It should be clearly labeled and frozen for later analysis.

4. Interview the patients as to the amount of food ingested, time course of symptomatology, origin of the material, and others who may have shared the meal. Contact these people immediately and collect samples from affected individuals.

5. Contact the Food and Drug Administration, Center for Food Safety and Applied Nutrition (Dr. Sherwood Hall, tel. (202)-205-4818, fax (202)-205-4881) immediately for further information and sample analysis, and archival.

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## CLINICAL RECOGNITION OF CIGUATERA FISH POISONING IN NEW CALEDONIA

by

Gilles ANGIBAUD (1)

**ABSTRACT.** - We retrospectively reviewed 56 cases admitted to Noumea hospital for serious manifestations of fish poisoning during five years (1991-1995). Cutaneous, gastrointestinal, benign neurological and cardiovascular symptoms were observed as commonly reported in endemic areas. The diagnosis of ciguatera fish poisoning was based on clinical grounds, specially the occurrence of mild neurological disturbances. Moreover, our study underlined 10 patients who experienced unfrequent and severe neurological signs, among whom two people presented neurological sequelae.

**RÉSUMÉ.** - Signes cliniques de la ciguatera en Nouvelle Calédonie.

Nous avons revu rétrospectivement les dossiers des patients admis à l'hôpital de Nouméa pour une intoxication ciguatérique entre 1991 et 1995. Cinquante-six patients (37 hommes, 19 femmes) constituent cet échantillon. Les 5 signes les plus fréquents furent: paresthésies (84%), diarrhée (82%), bradycardie (66%), vomissements (57%) et dysesthésies au contact de l'eau (55%). A côté de ces signes classiques, 10 patients présentèrent des signes neurologiques inhabituels et/ou sévères. Quatre eurent une évolution prolongée (dont 2 avec des séquelles neurologiques). Le diagnostic de la ciguatera reste essentiellement clinique, et les autres intoxications par fruits de mer présentant des tableaux cliniques proches sont revues.

**Key-words.** - Fish poisoning, New Caledonia, Ciguatera. Neurologic manifestations. Differential diagnosis, Prognosis, Mannitol.

Ciguatera fish poisoning is commonly encountered in intertropical areas. It is due to consumption of fish contaminated by ciguatoxins produced by a dinoflagellate: *Gambierdiscus toxicus* (Bagnis *et al.*, 1979; Withers, 1982). Ciguatera symptoms in humans are now well known by physicians and also by populations living in endemic areas. Gastrointestinal symptoms (abdominal discomfort, diarrhea), cutaneous symptoms (itching, pruritus) and cardiovascular symptoms (malaise, hypotension) are the most frequent signs. Even if ciguatera is rarely life-threatening or fatal, serious forms can occur, especially cardiovascular and neurological ones (Bagnis *et al.*, 1979; Glaziou and Legrand, 1994). The diagnosis depends on clinical grounds since no routine laboratory assay is usually available. Other types of seafood poisoning can mimic ciguatera clinical manifestations making diagnosis difficult (Eastaugh and Shepherd, 1989). We are going to present a series of patients admitted to Noumea General hospital with severe signs of suspected ciguatera poisoning.

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## MATERIAL AND METHODS

We retrospectively analyzed cases admitted to Gaston Bourret General Hospital (Nouméa, New Caledonia) during five years (1 January 1991-31 December 1995). In this series, diagnosis of ciguatera was made on clinical grounds. Two points were requested, i.e., history of fish eating and suggestive clinical signs. Laboratory testing for the presence of ciguatoxins was not available.

## RESULTS AND DISCUSSION

Fifty-six patients (37 males, 19 females) were recorded. They were mainly young (mean age 37 years, SD = 15, range 2-62). Eleven (20%) had already experienced prior episodes of ciguatera poisoning (numerous in 3 patients). Fish was identified in 46 cases (82%) with 41 carnivores (73%) and 5 herbivores (9%). The delay between the consumption of fish and the onset of clinical signs was known in 46 cases (82%). The mean delay was 11 hours with a wide range (1 h-6 d). The clinical manifestations are summarized in table I. The five more frequent signs were: paresthesias (n = 47), diarrhea (n = 46), bradycardia (n = 37), vomiting and nausea (n = 32) and dysesthesias (n = 31). Paresthesias are abnormal but not painful spontaneous cutaneous sensations. In ciguatera poisoning, they are commonly described by the patients as tingling or numbness in the extremities. On the contrary, dysesthesias are triggered by contact and are usually unpleasant or even painful. In ciguatera, itching and burning sensations, cold intolerance and reversal of temperature perception are the usual features of dysesthesias. Serious neurological features were not rare since they were found in 10 patients (18%). Six patients suffered polyneuropathy that is characterized by symmetrical distal sensory loss (mainly in lower limbs) and loss of tendon reflexes. Furthermore, muscle weakness was noted in 3 patients. Ataxia found in 5 patients means incoordination of movement and may be due to a lesion in the cerebellum or the sensory pathways. Ataxia was due to cerebellar involvement in our 5 patients. No death was noted. Most patients recovered fully before discharge. However, two patients (3.5%) with serious neurological features only slowly recovered: one with long lasting (2 months) cerebellar ataxia and polyneuropathy, the other one with polyneuropathy (45 days). Furthermore, two patients were discharged and then reviewed with persistent neurological disorders: one with ataxia and polyneuropathy, and the other one with both previous conditions and encephalopathy.

Table I. - Main clinical manifestations.

Gastrointestinal		Cutaneous and neurological		Cardiovascular		Serious neurological features: 10 (18%)	
Diarrhea	46 (82%)	Paresthesias	47 (84%)	Bradycardia	37 (66%)	Polyneuropathy	6 (11%)
Vomiting/nausea	32 (57%)	Dysesthesias	31 (55%)	Hypotension	23 (41%)	with weakness	3 (5%)
Abdominal cramping	24 (43%)	Muscle aches	20 (36%)			Ataxia	5 (9%)
		Pruritus	19 (34%)			Confusion	2
						Visual blurring	1

In the reported series of cases, the clinical manifestations observed were the ones commonly reported in ciguatera fish poisoning. Usually, they begin within 12 h after the consumption of fish. This is in accordance with observation by Bagnis *et al.* (1979) and Moulignier *et al.* (1995). Malaise and abdominal discomfort are often the first signs experienced by patients. Then, digestive features occur including abdominal pain, nausea, vomiting and diarrhea, followed by paresthesias and dysesthesias.

History taking is very important for the diagnosis of ciguatera poisoning since the notion of ingestion of a fish meal and suggestive clinical signs are the two main clinical grounds. As the clinical presentation depends on numerous factors including individual susceptibility, consumption of very highly contaminated species or parts of fish (viscera), increased sensitivity due to prior episode(s) and coingestion of other toxins (alcohol), the diagnosis is sometimes difficult in case of moderate intoxication. Cutaneous complaints are very suggestive and useful for diagnosis. Besides paresthesias, dysesthesias reported by patients are also very specific. So, these unpleasant or even painful contact-related sensations provide also an important help for diagnosis. Most of the times, any cutaneous contact (by clothes, sheets, ...) elicits dysesthesias, but contact to water is commonly unbearable leading to real water intolerance. As it was observed in our series of cases, reversal of temperature perception is largely reported by patients with cold intolerance.

Besides these "benign" symptoms of ciguatera poisoning, signs of severity occurring in serious forms that justify the hospitalisation are mainly cardiovascular and severe neurological manifestations. We diagnosed serious neurological features in 10 out of 56 patients (18%). This high percentage is quite uncommon. However, in this series the cases were only patients referred to the General Hospital in Noumea. They probably suffered more serious clinical signs than most cases which never had to be admitted to hospital. Various neurological manifestations have been reported: polyneuropathy and polyradiculoneuropathy, polymyositis, cerebellar ataxia, encephalopathy, blurred vision and vertigo are the most frequent (Allsop *et al.*, 1986; Stommel *et al.*, 1991; DeFusco *et al.*, 1993). Severe sequelae are not so rare in these neurological forms of ciguatera fish poisoning. Persistent sensory and/or motor disturbances in lower limbs, cerebellar ataxia or cognitive functions impairment have already been reported before this series (Allsop *et al.*, 1986; Stommel *et al.*, 1991). Similar neurological manifestations can be due to alcohol intake (i.e., polyneuropathy, encephalopathy, and cerebellar ataxia). Apart from its worsening effect on neurological symptoms of ciguatera poisoning, alcoholism on its own has also to be considered. No fatal case was noticed in our place during the 1991-1995 period. Fortunately, fatal cases of ciguatera fish poisoning are rare (Bagnis *et al.*, 1979; Allsop *et al.*, 1986).

Differential diagnosis with other seafood poisonings that can mimic ciguatera has to be made. Scombroid poisoning give gastrointestinal and general signs. Skin manifestations are different and classically described as flushing and hot sensations. Neurological disturbances are not seen in this condition. Symptoms begin earlier than in ciguatera poisoning, within minutes or a few hours after ingestion of fish. Tetrodotoxin poisoning is rather easily diagnosed on a clinical basis. Main features useful for differentiating from ciguatera are: species of fish eaten by the patients (puffer fish), the short delay between ingestion and onset (usually 15-30 min), the fastly worsening paralysis leading to tetraplegia with respiratory failure. Furthermore, after the acute (paralytic) phase requiring intensive care, the prognosis is quite good within a few days (Eastaugh and Shepherd, 1989). Other seafood poisonings like paralytic shellfish and domoic acid poisonings

have also to be considered. In fact, the kind of seafood meal, the course of symptoms, the delay between seafood eating and onset of clinical symptoms are the main data allowing a differential diagnosis.

The prognosis of ciguatera poisoning is generally good. However, this usually self-limited condition can leave patients with long-lasting fatigue and recurrent abnormal cutaneous sensations induced by ingestion of alcohol or non contaminated fish a long time after the end of the episode. Two out of 56 patients (3.5%) of this retrospective series had neurological sequelae.

The treatment of ciguatera poisoning is symptomatic. However, intravenous administration of mannitol (osmotic agent) was observed to provide dramatic reversal of ciguatera symptoms (Palafox *et al.*, 1988). Further prospective randomized studies are requested to assess this issue. Recent experimental results favoured this hope (Benoit *et al.*, 1996). If mannitol efficiency was confirmed, it would be fruitful in severe neurological ciguatera poisonings since the delay seems to be longer than in "common" forms (Angibaud and Rambaud, in press).

**Acknowledgements.** - The author wishes to thank Erika Mancel for her technical assistance in writing the manuscript and Sabine Rambaud for her help in collecting the data.

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## Workshop 6

### ADVANCES IN FISH BEHAVIOR STUDIES IN THE INDO-PACIFIC

Chairperson: **Ernst REESE**

- DONALDSON T.J. - Assessing phylogeny, historical ecology, and the mating systems of hawkfishes (Cirrhitidae). 789-796  
*Évaluation de la phylogénie, de l'histoire écologique et des systèmes de reproduction des Cirrhitidae.*
- FITZSIMONS J.M. & R.T. NISHIMOTO. - Application of fish behavior to stream monitoring on tropical pacific islands. 797-804  
*Utilisation du comportement des poissons pour la gestion des rivières des îles tropicales du Pacifique.*
- HOLBROOK S.J. & R.J. SCHMITT. - *In situ* nocturnal observations of reef fishes using infrared video. 805-812  
*Observations nocturnes in situ de poissons récifaux par vidéo infrarouge.*
- NISHIMOTO R.T. & J.M. FITZSIMONS. - Behavioral determinants of the in-stream distribution of native Hawaiian stream fishes. 813-818  
*Facteurs comportementaux de la distribution des poissons indigènes des rivières des îles Hawaï.*
- LOSEY G.S. & T.W. CRONIN. - The UV visual world of fishes. 819-826  
*Le monde visuel UV des poissons.*



## ASSESSING PHYLOGENY, HISTORICAL ECOLOGY, AND THE MATING SYSTEMS OF HAWKFISHES (CIRRHITIDAE)

by

Terry J. DONALDSON (1)

**ABSTRACT.** - Phylogenetic analyses of behavioral, ecological, and life history (BELH) characters have utility in testing hypotheses generated from systematic studies of morphological and molecular characters. Trees generated from BELH characters provide complimentary or alternate hypotheses of phylogeny within a group. BELH characters may also be examined by parsimony methods to determine which are historically constrained and which likely arise in response to environmental conditions. These analyses are applied to hawkfishes, protogynous reef-dwelling fishes with harem and facultatively monogamous mating systems. Here, phylogenetic relationships are outlined and an hypothesis describing the evolution of mating systems in relation to microhabitat utilization is proposed.

**RÉSUMÉ.** - Évaluation de la phylogénie, de l'histoire écologique et des systèmes de reproduction des Cirrhitidae.

Les analyses phylogénétiques incluant les caractères comportementaux, écologiques et biologiques (BELH en anglais) sont utiles pour tester les hypothèses proposées par les études systématiques fondées sur les caractères morphologiques et moléculaires. Les arbres obtenus en utilisant les caractères BELH proposent des hypothèses phylogénétiques complémentaires ou alternatives pour un groupe donné. Les caractères BELH peuvent aussi être examinés par les méthodes de parcimonie pour déterminer lesquels sont génétiques et lesquels apparaissent en réponse aux conditions environnementales (épigénétiques). Ces analyses sont appliquées aux Cirrhitidae, poissons de récif protogynes à système reproducteur de type polygame et occasionnellement monogame. Cette étude retrace les relations phylogénétiques et propose une hypothèse décrivant l'évolution des systèmes reproducteurs en fonction de l'utilisation de l'habitat.

**Key-words.** - Cirrhitidae, Mating system, Microhabitat use, Parsimony analysis, Reef fishes.

The use of phylogenetic methods to examine historical relationships between taxa and to trace the evolution of behavioral, ecological, and life history (BELH) characters has been the focus of recent studies of freshwater fishes (e.g., McLennan *et al.*, 1988; Mayden, 1992; McLennan, 1993, 1994, 1996). Unfortunately, similar effort is largely absent in studies of marine reef fishes. One difficulty contributing to this disparity is the relative paucity of BELH data for many marine and estuarine taxa, especially in the vast and highly diverse Indo-Pacific region. Patient collection of such data and subsequent analysis in a phylogenetic context will close this gap. Phylogenetic relationships among species of many reef-fish families have already been determined largely through studies of morphological or molecular characters. This knowledge provides the pattern by which the

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processes that govern the evolution of BELH characters may be explained (Brooks and McLennan, 1991) once these characters are defined. Here, I apply phylogenetic methods in an attempt to explain the evolution of mating systems in relation to microhabitat utilization in hawkfishes (Cirrhitidae). This family consists of at least nine genera and 33 species distributed largely in relatively shallow tropical and warm-temperate waters of the Indo-Pacific (Randall, 1963; Donaldson, 1986; Myers, 1991).

## MATERIALS AND METHODS

I constructed a phylogenetic tree based upon morphological characters given in Randall (1963, 1980), Lubbock (1977), and Lavenberg and Yanez (1972). I used MacClade

Table 1. - Data matrix for morphological characters of hawkfishes (Cirrhitidae). Genus abbreviations are: CHT: *Cirrhitus*; NA: *Neocirrhites*; PA: *Paracirrhites*; CTP: *Cirrhitops*; ISO: *Isocirrhites*; AMB: *Amblycirrhites*; CIR: *Cirrhitichthys*; CYP: *Cyprinocirrhites*; OT: *Oxycirrhites*. Outgroup is the Cheilodactylidae. 0: absent; 1: present; 2: 0 and 1.

Character	Outgroup	CHT	NA	PA	CTP	ISO	AMB	CIR	CYP	OT
1	1	1	1	1	1	1	1	1	1	1
2	0	1	1	1	1	1	1	1	1	1
3	0	1	1	1	1	1	1	1	1	1
4	0	0	0	0	0	0	0	0	0	1
5	0	0	0	0	0	0	1	1	0	1
6	0	1	1	1	1	1	0	0	1	0
7	0	0	0	0	0	0	0	0	0	1
8	1	0	1	0	0	0	0	0	0	0
9	2	1	1	1	1	1	1	1	0	1
10	0	0	0	0	0	0	0	0	1	0
11	1	1	1	0	0	0	0	0	0	1
12	0	0	0	1	1	1	1	1	1	0
13	0	1	0	0	1	1	1	1	1	0
14	1	1	0	1	1	1	1	0	0	0
15	0	0	1	0	0	0	0	1	1	1
16	1	0	0	0	0	1	1	0	0	0
17	1	0	1	0	1	0	1	1	1	1
18	0	1	0	1	0	0	0	1	1	1
19	0	1	0	1	0	1	1	0	0	0
20	0	1	0	0	0	0	1	1	0	0
21	0	0	1	0	0	0	0	1	0	1
22	0	0	0	0	1	0	0	0	0	0
23	0	0	0	0	1	0	0	0	0	0
24	0	0	0	0	0	0	0	0	1	0
25	0	0	0	1	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	1
27	0	1	1	0	1	1	1	1	1	0
28	0	0	0	1	0	1	1	1	1	0
29	1	1	1	1	1	1	1	1	1	0
30	1	0	0	0	1	0	1	1	1	2
31	2	2	1	2	1	0	1	1	1	1

vers. 3.01 (Maddison and Maddison, 1992) to analyze, with parsimony methods, the character matrix (Table I) of 31 unordered characters for nine genera (Appendix A). Because this tree was constructed from external morphological characters only, and because the family is in need of revision (J.E. Randall, pers. comm.), it is provisional. Taxa were limited to genera because of incomplete knowledge of BELH characters for many species that restricted later efforts to trace character evolution. The resulting tree presented here was deemed the shortest, and thus most parsimonious of all possible trees generated (Maddison and Maddison, 1992).

Morphological characters for hawkfishes were polarized using the putative sister group, the Cheilodactylidae, as the outgroup. Sufficient morphological data for this family of cirrhitoid fishes were extracted from Allen and Heemstra (1976), and Randall (1983). Relationships within the superfamily Cirrhitioidea are currently being examined on a molecular level (C. Burrige, pers. comm.).

For this paper, I focused upon the evolution of microhabitat utilization (four characters) and mating system (two characters). Characters (Table II) were derived from Donaldson (1986, 1987, 1989, 1990), Donaldson and Colin (1989), Tanaka and Ohyama (1991), Tanaka and Suzuki (1991), Tanaka (1995), Demartini (1996) and Demartini and Donaldson (1996). Outgroup characters were derived from Tong and Vooren (1972), Sano and Moyer (1985), and McCormick (1989). I mapped characters (Table III) on this tree following methods described in Brooks and McLennan (1991), and Maddison and Maddison (1992). Then, I developed an hypothesis describing the evolution of mating systems in relation to patterns of microhabitat use.

Table II. - Microhabitat use and mating system characters traced in a phylogenetic analysis of hawkfishes (Cirrhitidae).

Character	Character type	No.
Substrate-dwelling	Microhabitat use	1
Water column-dwelling	Microhabitat use	2
Facultative coral-dwelling	Microhabitat use	3
Obligate coral-dwelling	Microhabitat use	4
Haremic mating group	Mating system	5
Facultative monogamy	Mating system	6

Genus	Coding
Cheilodactylidae	100010
<i>Cirrhitus</i>	100010
<i>Neocirrhites</i>	001011
<i>Paracirrhites</i>	010010
<i>Cirrhitops</i>	100010
<i>Isocirrhites</i>	100010
<i>Amblycirrhites</i>	100010
<i>Cirrhitichthys</i>	110010
<i>Cyprinocirrhites</i>	000110
<i>Oxycirrhites</i>	001011

Table III. - Coding sequence of microhabitat and mating system characters in hawkfish genera (Cirrhitidae) (see Table II for character descriptions).

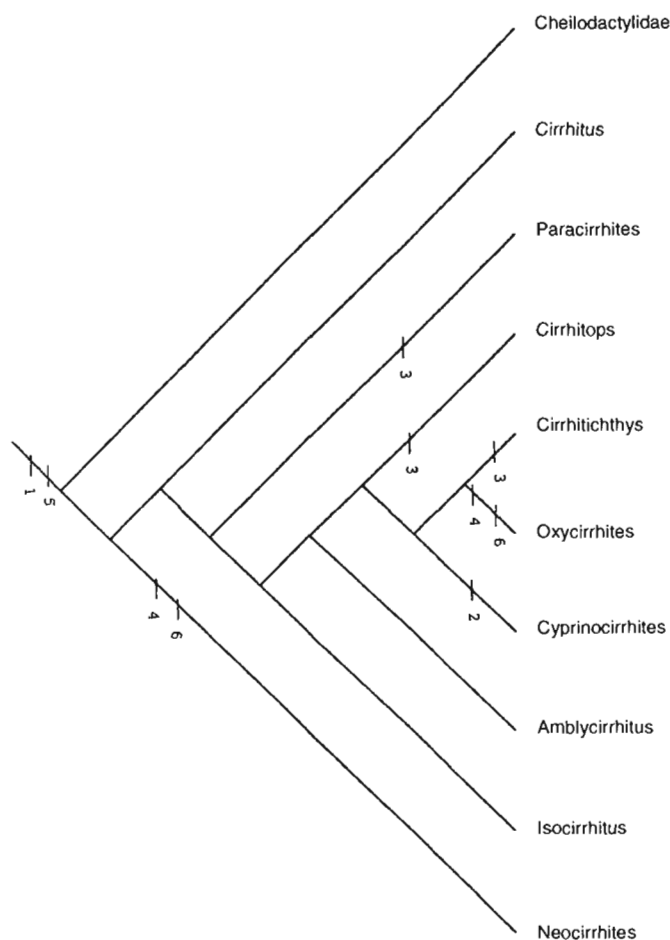


Fig. 1. - Cladogram describing provisional phylogenetic relationships of nine genera of hawkfishes (Cirrhitidae) based upon parsimony analysis by MacClade (Maddison and Maddison, 1992) of 31 unordered morphological characters. Character reconstructions of microhabitat use (1-4) and mating system (5-6) are mapped on this tree. Characters are: 1) substrate-dwelling, 2) water column-dwelling, 3) facultative coral-dwelling, 4) obligate coral-dwelling, 5) harem mating group, and 6) facultative monogamy.

## RESULTS AND DISCUSSION

A provisional phylogenetic tree for hawkfish genera is given in figure 1. Tree statistics are as follows: tree length = 57; consistency index = 0.58; retention index = 0.49. Doubtless, these measures will improve following systematic revision of the family and construction of a new tree. On this tree, the most parsimonious character reconstructions for microhabitat utilization and mating system have been mapped (Fig. 1). Substrate-dwelling and harem mating systems are ancestral to this family and the outgroup. These characters are retained in *Amblycirrhites*, *Cirrhitus* and *Isocirrhites* (Randall, 1963; Donaldson, 1987, 1990), and are considered plesiomorphic. Substrate-

dwelling is likely retained because of one or more factors, including relatively large body size in some species, large territory size in some species, the ability to find shelter in a variety of structures, cryptic coloration, and cryptic (Donaldson, unpubl. data) or possibly nocturnal behavior in some species (see Randall, 1963). These characters remain to be examined between species (Donaldson, unpubl. data). In substrate-dwelling, access to potential mates is not limited by relative isolation or size of microhabitat, as one might find if microhabitat utilization is confined to a relatively small, discrete unit, such as a coral head (Donaldson, 1989). So, the ancestral harem mating system is retained.

Water column utilization, a departure from substrate-dwelling, is a derived character found only in the monotypic genus *Cyprinocirrhites*, and is considered derived. A harem mating system has been retained in this genus. Members of mating groups often seek shelter in common holes in the substratum (Donaldson, unpubl. data). This behavior likely promoted retention of the ancestral harem mating system since access to mates is not microhabitat-limiting. Facultative coral utilization occurs in *Cirrhitichthys*, *Cirrhitops* and *Paracirrhites*. Some members of these genera utilize shrub corals as microhabitat but may also use other forms of microhabitat, including coral boulders, rock, and the substratum (Donaldson, 1990, unpubl. data). *Cirrhitichthys oxycephalus* and *Cirrhitops fasciatus* are two examples where this behavior is an exception to substrate-dwelling observed in other species of these genera (Randall, 1963, 1980; Thresher, 1984; Donaldson, 1990, unpubl. data). In *Paracirrhites*, all species utilize shrub corals to some extent, although not exclusively (Randall, 1963; Donaldson, 1990, unpubl. data; DeMartini and Donaldson, 1996; DeMartini, 1996). This character is derived from the ancestral condition. Relative body size, feeding preferences, coral size and the utilization of corals as courtship and spawning rendez-vous sites, and sheltering sites, all likely influenced this derivation (Donaldson, 1990, unpubl. data; DeMartini, 1996). Homoplasy, in that convergent or parallel evolution of this character may have occurred, may be indicated since this character is absent from both *Cyprinocirrhites* and *Oxycirrhites*. Alternately, the patterns of microhabitat utilization for these species may have evolved from facultative coral-dwelling, although this possibility seems more likely in *Oxycirrhites* than in *Cyprinocirrhites*. A conservative approach, that invokes Hennig's Auxiliary Principle (cited in Brooks and McLennan, 1991: 34), is to not assume convergent or parallel evolution but rather assume homology until a more precise phylogenetic tree is produced that could elucidate hawkfish relationships more clearly. With respect to mating system, access to mates is again not microhabitat-limited and so the ancestral harem mating system is retained (Donaldson, 1990).

Obligate coral-utilization occurs in *Neocirrhites* and *Oxycirrhites*, both monotypic genera. The former genus lives in shrub-like corals of the genus *Pocillopora* (Donaldson, 1990, unpubl. data) whereas the latter lives in gorgonian and antipatharian corals (Donaldson and Colin, 1989; Donaldson, 1990, unpubl. data). This character is considered derived from the ancestral state of substrate-dwelling. Both genera also exhibited facultative monogamy (Donaldson, 1989). Here, the mating system is constrained by microhabitat size or distribution and is thus microhabitat-limited. Therefore, an effective mating group consists of a male-female pair instead of the plesiomorphic character state in which the system is a male-dominated harem. If the microhabitat is large enough to accommodate more than a pair, or is close enough to another coral, and hence another potential mate, then harem formation can occur. This possibility is uncommon, however (Donaldson, 1989). Thus, facultative monogamy likely arose as an adaptation to obligate coral-dwelling in these two genera. Alternately, obligate coral-dwelling and facultative

monogamy occurred in all genera but was lost in all but *Neocirrhites* and *Oxycirrhites*. This hypothesis is much less parsimonious, because the number of additional steps required (up to 18) is greater.

**Acknowledgements.** - My thanks to E. Reese for his kind invitation to participate in the workshop "Advances in Fish Behavior Studies in the Indo-Pacific" held at the 5th Indo-Pacific Fish Conference, Noumea. I am grateful to H. Aratake, C. Burridge, M. Fitzsimons, and a host of others for past help or collaborative effort in the study of hawkfishes. Special thanks are extended to J. Randall and A. Nakazono for their tireless assistance. I am grateful for the comments of an anonymous reviewer. This paper was written while I was a Japanese Society for the Promotion of Science-National Science Foundation Fellow in the Department of Fisheries, Kyushu University. This is Contribution Number 400 of the University of Guam Marine Laboratory.

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## Appendix A. - Morphological character descriptions.

1. Scales cycloid.
2. Two flattened spines on the opercle.
3. Three to five rows of scales above the lateral line.
4. Snout elongate.
5. Snout pointed.
6. Snout blunt.
7. Body slender.
8. Body deep and compressed.
9. Caudal fin rounded, truncate or slightly emarginate.
10. Caudal fin lunate.
11. Small scales on cheek
12. Large scales on cheek.
13. Palatine teeth present.
14. Upper margin of preopercle finely serrate or smooth.
15. Upper margin of preopercle coarsely serrate.
16. Lower 5 pectoral rays unbranched.
17. Lower 6 pectoral rays unbranched.
18. Lower 7 pectoral rays unbranched.
19. Soft dorsal rays 11.
20. Soft dorsal rays 12.
21. Soft dorsal rays 13.
22. Soft dorsal rays 14.
23. Soft dorsal rays 15.
24. Soft dorsal rays 16.
25. Single cirrhii from membrane at tip of each dorsal spine.
26. Cirrhii as 2-4 from membrane at tip of most dorsal spines.
27. Cirrhii as a tuft from membrane at tip of each dorsal spine.
28. Interorbital scaled.
29. Preorbital with hind margin not free.
30. Pectoral fins extend to the origin of the pelvic fin.
31. Pelvic fins extend to or beyond anus.

## **APPLICATION OF FISH BEHAVIOR TO STREAM MONITORING ON TROPICAL PACIFIC ISLANDS**

by

J. Michael FITZSIMONS (1) & Robert T. NISHIMOTO (2)

**ABSTRACT.** - In Hawai'i, the social behavior of indigenous stream fishes (Gobiidae and Eleotridae) occurs as sequences of discrete movements and postures that can be readily observed in the islands' clear mountain streams. These units of behavior are uniform within a species, and the sequence in which they appear in a given social context is consistent throughout the chain of high islands. Stereotypy in behavior enables investigators to use ethological studies for island fishes as a valuable adjunct to stream surveys; the information addresses directly whether or not the animals are engaging in activities required for maintenance and reproduction. Behavioral data can be better indicators of stream degradation and more useful in judging whether stream restoration has been successful than are simple lists of species and numbers of animals. We suggest that procedures for applying behavioral information to stream monitoring in Hawai'i can be extended to the other islands of the tropical Pacific where members of the same two fish families form the major faunal component of island streams.

**RÉSUMÉ.** - Utilisation du comportement des poissons pour la gestion des rivières des îles tropicales du Pacifique.

Dans les rivières de montagne des îles Hawaï, le comportement social des poissons indigènes (Gobiidae et Eleotridae) montre des périodes d'activité et de postures qui peuvent être facilement observées grâce aux eaux claires de ces rivières. Ces types de comportement sont uniformes pour une espèce donnée, et la période au cours de laquelle ils ont lieu est la même pour l'ensemble des îles de l'archipel. Ces comportements stéréotypés permettent aux aménageurs d'utiliser les études éthologiques sur ces poissons insulaires comme un appoint précieux dans la surveillance des rivières. Ces données permettent directement de savoir si les poissons sont engagés dans des activités d'alimentation ou de reproduction. Ainsi, les données comportementales peuvent être de bien meilleurs indicateurs de la dégradation des rivières, et aussi bien plus utiles, pour évaluer la réussite d'une réhabilitation de rivière, que de simples listes d'espèces et d'effectifs. Nous suggérons que ces méthodes utilisant l'information sur le comportement des poissons comme élément de la gestion des rivières à Hawaï, puissent être étendues aux autres îles tropicales du Pacifique où des espèces de ces deux mêmes familles constituent la majorité des composants faunistiques des rivières insulaires.

**Key-words.** - Gobiidae, Eleotridae, ISE, ISEW, Hawai. Pacific islands, Freshwater fishes, Behavior, Biomonitoring.

Freshwater fishes on oceanic islands of the tropical Pacific are common in perennial streams fed by orographic rain on windward slopes. The animals belong mostly to two closely related families, the Gobiidae and Eleotridae. These families are largely ma-

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rine, but both include species adapted for life in fresh water. Most freshwater gobioids have an amphidromous life cycle characterized by two migrations, one when newly hatched larvae are washed from the stream into the sea and the other when the animals return to freshwater streams some months later (Radtke *et al.*, 1988). The recruits enter stream mouths as larvae or recently transformed postlarvae, and species differences in upstream movement by the young fishes determine the final distribution of adults along the length of the stream (Tate, 1996). Among the five species of gobioid fishes living in Hawaiian fresh waters, complex social behavior is well developed in the gobies but is limited to reproduction (territoriality and courtship) in the one eleotrid species (Fitzsimons and Nishimoto, 1991). In a collaborative effort by researchers from the Hawai'i Division of Aquatic Resources and the Louisiana State University Museum of Natural Science, changes in the social behavior of stream fishes proved valuable in a 20-month study assessing initial effects and tracing recovery of streams on Kaua'i after that island was struck by Hurricane Iniki on 11 September 1992 (Fitzsimons and Nishimoto, 1993, 1995, 1996). Since the hurricane study, behavioral observations have become an integral part of stream surveys conducted by the Division of Aquatic Resources. In this report, we wish to describe certain gear and procedures used in Hawaiian stream studies, and, with examples based on Hawaiian species, suggest how the scope of surveys in other island groups can be enhanced by adding an aspect that focuses specifically on behavioral adaptations essential for survival by native stream fishes.

## METHODS

### Recommended equipment, supplies and procedures

#### Field gear

A heavy duty, full-length wet suit is required to allow the underwater observer to lie quietly in water at temperatures 15-25°C. A two-piece "Farmer John" style is ideal because the jacket can be removed to prevent overheating when hiking to and from the study site. Elbow and knee pads are recommended. In cold water, a hood, liner, and gloves are essential. Gloves with Kevlar™ palms and fingers are helpful in gripping smooth rocks and protecting hands from sharp edges. Footwear should include the felt-soled Japanese tabi or fly-fishing boots. Typical diver's booties or other hard-soled shoes and boots will slip on wet, algae-covered rocks. Swim fins are seldom useful except in deep splash basins below water falls. Although the gear described here may seem cumbersome, it is important for preventing the diver from becoming chilled and for protection from cuts, scrapes, and bruises during inevitable falls on slippery surfaces. A 30 m length of nylon rope, thick enough for a comfortable grip, is valuable for safely rappelling without a harness down a moderately steep slope to reach a stream. The free end of the rope is passed between the climber's legs from the front, draped over the left shoulder, and gripped with the left hand at or below waist level; the standing (attached) section of the line is grasped with the right hand at about chest level. As the climber descends, slack in the rope is created with the left hand and controlled by the right hand. A net bag or backpack with shoulder and sternum straps is practical for carrying loose gear while leaving both hands free for climbing. A dive mask, glasses, camera, tape recorder, tape, film, etc. should be wrapped separately in a heavy, cushioning cloth (towel) and placed in crush-proof boxes before being carried in the backpack. Rubbermaid™ bread boxes and refrigerator containers that seal tightly are usually satisfactory, but items that must stay completely dry should be sealed inside two

or more thick plastic bags before being placed in boxes. A hand towel stored in a heavy plastic bag is needed to dry hands before handling a tape recorder or camera that is not waterproof.

### Recording observations

Notes are written underwater on a waterproof slate (such as a sheet of white or light-colored fiberglass) or on spiral-bound Mylar™ sheets held to the slate with large rubber bands. Semi-loose sheets are preferred because they can be detached and filed as backup notes. The lead and eraser of a No. 2 pencil attached to the slate with a heavy cord work well underwater on both types of writing surfaces, and the pencil is sharpened easily wet or dry with a diver's knife. At convenient time intervals, the diver leaves the water and reviews underwater data while speaking into a hand-held tape recorder. Recordings are later transcribed (preferably daily) into a field notebook or word processor.

### Selection of study sites

Stream morphology and distance inland can be used to divide the stream into discrete study areas. In Hawai'i, surveys rely on distinguishing between lower, middle, and upper reaches of streams. Lower reaches include the section of the stream from the mouth inland until the first barrier to migration (usually a waterfall) is encountered by those species of fishes unable to climb or go around the obstruction. These fishes include eleotrids (*Eleotris sandwicensis*), certain freshwater gobies (*Stenogobius hawaiiensis*), and marine species that occasionally enter fresh water as adults (mulletts, jacks, threadfin, sharks, and others) or are common there as juveniles (*Kuhlia sandwicensis*). Middle and upper stream reaches include mostly the "true gobies" that have a well-developed sucking disk formed by the fusion of the pelvic fins. The ventral mouth of *Sicyopterus stimpsoni* is used in conjunction with the pelvic disk while climbing "inch-worm fashion" (Schoenfuss *et al.*, 1997). At some distance inland, a steeper gradient, fewer large waterfalls, and a profile of long stretches of "stair-step cascades" usually distinguish the upper reach of a stream from the middle section. In Hawai'i, *Lentipes concolor* is often the only fish species in upper section of streams (Nishimoto and Fitzsimons, 1986; Kinzie, 1988), but, in streams with a more gradual slope, *L. concolor*, *S. stimpsoni*, and *Awaous guamensis* may be found in the same stretch (Nishimoto and Kuamo'o, 1991). However, in such streams, *L. concolor* typically perches on the upper surface of rocks and boulders, *S. stimpsoni* is most abundant in shallow, swift riffles, and *A. guamensis* occurs in loose aggregations along the bottom in the main channel where current velocity is sharply reduced (Fitzsimons and Nishimoto, 1990). Thus, even when the fishes occur together in a section of stream, there is discernible separation of species.

### Use of indicator species

With a mere five species of fishes comprising the entire stream-fish fauna in Hawai'i and given their usual species-specific distribution along the length of a stream, the designation of one or two species for judging the "health" of a stream appears unnecessary. Elsewhere in the Indo-Pacific where the number of stream fishes may be as two or more times higher (Nelson *et al.*, 1995), selection of one or two species representing each major habitat type may be a practical procedure. A species might be selected as an indicator species because it is common and abundant in a discrete type of habitat or, instead, species selection might focus on a fish whose limited occurrence indicates more stringent

habitat requirements and apparent vulnerability to change. Ideally, the decision will result in both types of species indicated for concentrated study.

### Types of behavior suitable for enhancing surveys

The behavior of fishes and other lower vertebrates is largely genetically determined. If appropriate environmental conditions are available to the animals during ontogeny, their behavior is largely adaptive, stereotypical, and predictable. Although there are many exceptions, learning does not have a major influence in determining the behavioral repertoire of adult fishes. Thus, discrete units of behavior are often easier to identify in fishes, and they are less likely to vary within a population. Even though experience is less significant in affecting behavior of fishes than in birds and mammals, the behavior of fishes is not necessarily less complex or of fewer types than seen among higher vertebrates. For this reason, a complete cataloguing of the behavior of a stream-fish species during its entire life cycle, while perhaps instructive, would not be practical as an adjunct to faunal surveys because of time constraints. Instead, we recommend that the survey team emphasizes those behaviors that are most important to the survival of the individual animal and ultimately the species to which it belongs. Maintenance (food-getting) and reproduction (territoriality, courtship, and spawning) are the suggested focus.

## RESULTS

Although most gobies and eleotrids are carnivores, in fresh water some species have become obligate herbivores while others feed both on algae and invertebrates. Herbivores feed more frequently, and bouts of feeding are of longer duration than observed for carnivorous stream fishes. When not engaged in courtship or territorial defense, *Sicyopterus stimpsoni* appears to feed nearly continuously by scraping diatoms and small pieces of filamentous green and blue-green algae (Kido, 1996a) from rocks and boulders with brushlike movements of long bifid teeth in the upper jaw (Schoenfuss *et al.*, 1997). Conspicuous, scallop-edged patches on boulders are a clear indication that fish of this species are present in the stream and are feeding (Fitzsimons *et al.*, 1993). *Lentipes concolor*, a species whose diet includes mostly algae and aquatic invertebrates (fly larvae, oligochaetes) (Kido, 1996b), usually occupy conspicuous perches along the length of the stream. Although the fish make occasional stabbing bites at the substrate, they often launch themselves from a perch to intercept potential food items drifting downstream. The one other upstream species, *Awaous guamensis*, and the downstream goby *Stenogobius hawaiiensis* also leave the bottom to pick individual food items drifting in the water column, but fishes of these two species feed most often by taking large bites of loose substrate and passing the material through their "pharyngeal mill". While food is being removed inside the oral cavity of the fish, sand and small pieces of gravel literally flow out from under the lower edge of the operculum. *Eleotris sandwicensis* is a sit-and-wait predator. Although actual feeding has been observed infrequently, the orientation of the fish in a stream place them in ideal positions for capturing smaller fishes and invertebrates. During peaks in the migration of larval gobioid fishes from the ocean into streams, for example, *E. sandwicensis* sometimes aggregate in large numbers at the mouths of streams where the gut may become distended from ingesting small fishes. In Hawai'i, larval eleotrids gain access to fresh water mostly at night when conspecific adults are not feeding (Nishimoto and Kuamo'o, 1997).

With the exception of *A. guamensis* (Ha, 1991; Kido and Heacock, 1991), Hawaiian stream fishes spawn all year round with a peak in summer months (Fitzsimons and Nishimoto, 1991). Again with a single exception (*S. hawaiiensis*), these fishes exhibit conspicuous territorial behavior in guarding feeding sites (*S. stimpsoni*), clusters of eggs (*A. guamensis*), or larger areas encompassing spawning sites (*L. concolor*, *S. stimpsoni*, *A. guamensis*, and *E. sandwicensis*) (Fitzsimons and Nishimoto, 1991). In all five species, striking color changes and species-specific movements and postures, most conspicuous in males, indicate clearly that the animals are engaged in courtship, spawning, or territorial defense.

#### Techniques for observing, recording, and scoring behavior

Streamside observations may be possible by using polarized glasses or binoculars with a short focal length, but unobstructed viewing is usually improved markedly by the investigator joining the fishes in the stream. The observer enters the stream as quietly as possible and moves slowly against the current until the section of stream known to have fishes or likely to have fishes comes into view. By limiting movements in a generally upstream direction, silt, leaf litter, and other debris lifted up by the observer will be swept downstream without obscuring vision. If underwater visibility is less than about two meters, observations will be difficult or impossible. Visibility of three or four meters is usually sufficient, but greater distances are ideal. It is critical that the observer remain completely motionless in the water for the fishes to resume usual activity. For watching some species, the swimmer may be required to observe from behind a large boulder or other natural "blind". For some species, too, it may be necessary to leave the underwater slate onshore and to keep hands and feet tucked in close to the body to avoid frightening the fishes. For particularly skittish animals, a diver can place only his face into the lip of a small waterfall in order to watch fish immediately upstream. In streams frequented by carnivorous birds (e.g., black-crowned night-heron *Nycticorax nycticorax*) and spear-fishermen, a biologist should expect the fishes to be unusually wary.

Two safety precautions cannot be overemphasized. A diver with eyes and ears covered by water usually will not be able to see or hear a waterfall until he or she has come within a few feet of its edge. Once in the chute immediately upstream from a large waterfall, the person may not be able to find a hand- or foot-hold in the smooth substrate and will be swept over the precipice. Flash floods are characteristic of streams on oceanic high islands. On small islands or those with steep slopes, the diver may be able to see the approach of storms or the onset of localized heavy rainfall that contributes to the headwaters of a study stream. However, on low, heavily eroded islands or where the rainforest blocks view of the mountains, a diver's first indication of danger may be the sound of automobile-size boulders being overturned by a wall of water rushing downslope. Advance warning of a flash flood, if it occurs, often includes a rise in water level and a decrease in underwater visibility. These changes may occur slowly at first, so it is essential that a diver identify or place an object close to the waterline and check it periodically. Even a slight decrease in visibility during an observation period probably indicates that a stream has left its banks at an upstream location and is beginning to wash soil and debris into the main channel. The diver should leave the water immediately and seek higher ground. A wait-and-see attitude could result in loss of valuable equipment, injury, and death.

Before behavior studies can be part of routine stream surveys (Baker, 1991; Baker and Foster, 1992), the individual with this responsibility must spend sufficient time in the water to become familiar with the habits of selected fishes in each section of a stream

where species lists and counts are to be made. Although the behaviors during feeding, territorial defense, courtship, and spawning have species-specific features, they are sufficiently similar within gobioid fishes generally that learning them in one species will shorten the time needed to recognize them in others. For this reason, reviewing published descriptions of the behavior in related species of island fishes before beginning field studies is likely to be helpful. Against this background, the underwater observer will soon be able to associate a bout of behavior with the appropriate context (such as feeding, courtship, territoriality, dominance, etc.), and that information in itself may be sufficient for scoring whether or not the activity of a species is typical. The next step for more detailed analysis is being able to discern the individual units (discrete movements and postures) that collectively comprise a bout of behavior. Even complex interactions, when viewed repeatedly, can be broken down into individually identifiable units. Among fishes, behavioral units are typically stereotyped and easily recognized among members of the same population (Lorenz, 1970). In Hawaiian stream fishes, the units of behavior common in feeding and social interactions within each of the five species are identical between islands (Fitzsimons and Nishimoto, 1991), and, except during extreme disturbances (such as a hurricane), even the sequences in which the behaviors occur do not vary significantly between populations on islands 384 km apart (Hawai'i and Kaua'i) (Nishimoto and Fitzsimons, 1986; Fitzsimons and Nishimoto, 1991; Fitzsimons *et al.*, 1993). Once a biologist is able to recognize the color patterns and stereotyped postures and movements associated with, say, courtship in a certain species, that person will have the capability of seeing just a portion of the behavioral repertoire and know that, in this example, reproductive activity is indeed occurring at that time among fish of this species in a certain study stream. Thus, when the individual becomes a "self-trained observer", he or she will be able to spend a relatively short time in the water and score whether the study species is exhibiting typical or abnormal behavior during feeding and social interactions.

## DISCUSSION

It is not the intent of this report to suggest that behavior studies can replace traditional survey techniques in streams on oceanic islands. Assaying species composition and determining the distribution and relative number of individuals is an irreplaceable first step in estimating the "biological well being" of a stream, in tracking changes over time, and, ultimately, in comprehending causes of change at the level of the ecosystem of which the stream is one component. Species lists and counts are the beginning point, too, for determining whether the size of adult populations is recruitment- or resource-limited, for understanding predator-prey relationships, for revealing how changes in water level affect aquatic animals, and for any number of other studies that contribute directly to understanding the natural history of stream animals. Faunal surveys form the data base upon which rests recommendations for conservation, management, restoration, and mitigation. However, information on the behavior of stream animals can provide critical information not otherwise available. Shortly after Hurricane Iniki devastated the Island of Kaua'i, standard visual surveys revealed alterations in species composition and instream distribution of indigenous stream fishes (Fitzsimons and Nishimoto, 1993), but disruptions in normal feeding behavior, courtship, and spawning in all species, and a striking increase in aggressive interactions in one species were better indicators for assessing relative damage in three study streams. Although the greatest rate of recovery occurred within four months,



behavioral observations emphasizing maintenance and reproduction during the 20-month study revealed a gradual restoration of the stream community that would not have been detected by using only fish surveys and physicochemical data (Fitzsimons and Nishimoto, 1995).

Gobies and eleotrids are ubiquitous in freshwater streams on oceanic islands throughout the tropics. One Hawaiian species, *Awaous guamensis*, ranges to the islands of the Western Pacific, and the other species, although endemic to Hawai'i, have congeners or close relatives throughout the basins of the Pacific and Indian oceans where orographic rain or precipitation associated with weather patterns on nearby continents ensures year-round flow in island streams. We expect the behavior of stream fishes in other island groups to have patterns similar to those observed for Hawaiian fishes, but their species-specific differences in behavior probably will provide the first and best line of evidence for understanding how a much larger number of related species are able to exist together in an island stream. The same information may also have practical value in detecting disturbances, whether natural or manmade, and in suggesting ameliorative action.

**Acknowledgements.** - The facts, inferences, and hypotheses presented in this paper are based on a series of studies that would not have occurred without the continuing counsel and encouragement of William S. Devick, Division of Aquatic Resources, Department of Land and Natural Resources, State of Hawai'i. The research was funded for Nishimoto in connection with his employment in the Division of Aquatic Resources and for Fitzsimons via a grant from the Division to his university through the Research Corporation of the University of Hawai'i (Dingell-Johnson/Wallop-Breaux Sport Fish Restoration Project F-14-R-18).

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## IN SITU NOCTURNAL OBSERVATIONS OF REEF FISHES USING INFRARED VIDEO

by

S.J. HOLBROOK & R.J. SCHMITT (1)

**ABSTRACT.** - *In situ* video using infrared illumination enabled observation and recording of behavior of reef-associated fishes during the night as well as the day without the confounding effects from the presence of human observers. The camera was positioned on a reef and power was supplied from shore over a coaxial cable, which also returned the video signal to the laboratory where images were recorded on videotape. The remotely operated video system was used to investigate sources of mortality of newly-settled individuals of *Dascyllus trimaculatus*, the three-spot dascyllus, while associated with their host anemones. Observations revealed that several types of predators, including a variety of piscivorous fish and octopus, frequently visited anemones throughout the diurnal cycle. Although attacks on young damselfish occurred during the day as well as night, level of activity of predators was higher at night.

**RÉSUMÉ.** - Observations nocturnes *in situ* de poissons récifaux par vidéo infra-rouge.

L'utilisation *in situ* d'une caméra vidéo infrarouge a permis d'observer et d'enregistrer, sans le regard gênant d'observateurs humains, le comportement de poissons récifaux pendant la nuit aussi bien que pendant la journée. La caméra était placée sur un récif et alimentée par un câble électrique provenant de la côte. Ce système a permis de recevoir le signal vidéo au laboratoire où les séquences étaient enregistrées sur vidéocassettes. Ce système vidéo télécommandé a été utilisé pour déterminer les causes de mortalité de la demoiselle à trois taches, *Dascyllus trimaculatus*, associée aux anémones de mer. Divers prédateurs ont été observés dont plusieurs poissons piscivores et des poulpes qui visitaient fréquemment les anémones durant leur cycle journalier. Les jeunes poissons-demoiselles sont attaqués de jour comme de nuit, avec une fréquence des attaques plus élevée la nuit.

**Key-words.** - Pomacentridae, *Dascyllus trimaculatus*, Reef fish, Behavior, Predation, Underwater video, Infrared illumination.

The abundance of demersal fishes on reefs is influenced by several processes that operate at different times and places (Fig. 1). Events that affect numbers of planktonic larvae as well as their transport to reefs can be crucial, because these determine the supply of settlers (Doherty and Williams, 1988; Doherty, 1991; Doherty and Fowler, 1994a, 1994b). The process of settlement, which typically occurs at night, is rarely observed directly (but see Holbrook and Schmitt, 1997), and virtually nothing is known about the activities and fates of fishes during and just following the transition from the plankton to the reef. It is possible that strong interactions with conspecifics (Sweatman, 1983, 1985a; Forrester, 1995; Elliott *et al.*, 1995; Schmitt and Holbrook, 1996, 1999; Holbrook and Schmitt, 1997), predators (Doherty and Sale, 1985; Holbrook and Schmitt,

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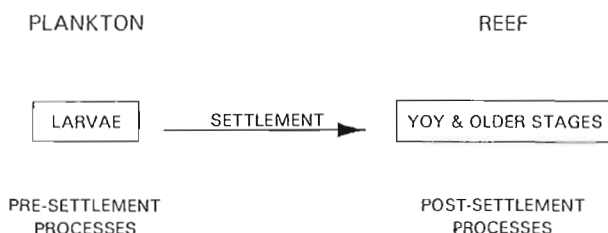


Fig. 1. - Diagrammatic representation of events during the early life stages of reef fish.

1997), or competitors (Jones, 1987a, 1987b, 1991; Sweatman and St. John, 1990) occur during or just after settlement, but at the present time too little is known to judge their effects on abundance. The lack of information about biotic interactions during the critical early period on the reef has prevented an assessment of the relative importance of physical and biotic processes in shaping patterns of larval supply and subsequent recruitment of demersal fish.

The study of the early post-settlement life of reef fishes has been hampered in part because obtaining behavioral information about young reef fishes is extremely difficult. During the daytime when observations by divers are possible, the presence in the water of human observers often significantly alters behavior of the fishes or their predators. And at night, when crucial events occur, darkness usually precludes behavioral study. These logistic constraints have slowed the progress of behavioral studies of reef fish. Here we describe *in situ* video technology with infrared illumination that enables observation and recording of behavior of reef-associated fishes during the night as well as the day. The use of this approach circumvents several of the major problems encountered with current observational approaches. In this paper we illustrate how the video technology can be utilized to study behavior of early post-settlement stages of *Dascyllus trimaculatus*, the three-spot dascyllus. This species is a common diurnal planktivore that occurs throughout the tropical Indo-Pacific (Bagnis *et al.*, 1987; Fautin and Allen, 1992), normally living in association with large sea anemones. Below we describe interactions between newly-settled three-spot dascyllus and their potential predators, with the goal of better understanding sources of early mortality.

## MATERIAL AND METHODS

Eggs of *Dascyllus trimaculatus* are laid on benthic substrata and fertilized externally, larvae hatch and enter the plankton after ~3 days (Garnaud, 1957; Fricke, 1974; Thresher, 1984), and young settle at night after 22-24 days (Wellington and Victor, 1989; Kingsford *et al.*, unpubl. data). *D. trimaculatus* settle almost exclusively on large sea anemones (Fautin and Allen, 1992) where they reside in groups before becoming free-ranging adults at a body size between 60-70 mm SL. The daily cycle of *D. trimaculatus* includes active feeding in the water column above the anemone from dawn to dusk. The largest juveniles often move a meter or more into the water column from the anemone while feeding. The fish are relatively inactive at night and remain in close contact with the anemone, usually among the tentacles (Holbrook and Schmitt, 1997). In Moorea, French Polynesia (Galzin and Pointier, 1985), the location of the present study, the sea anemone commonly occupied by *D. trimaculatus* is *Heteractis magnifica* (whose taxo-

onomic status is under review; Fautin, pers. com.). We studied behavior of newly-settled damselfish on anemones that we transplanted to depths of up to 6 m on a reef offshore of the University of California Berkeley Gump Biological Station on the north shore of Moorea, FP (17°30'S, 149°50'W). Each anemone was placed on natural reef substrate ~5 m from its nearest neighbor. Anemones used in the observations ranged in size from 20 x 20 cm to 30 x 30 cm and contained up to ten damselfish. The three-spot dascyllus ranged in age from new settlers up to ten days post-settlement, with body sizes of 10-15 mm SL.

### Video and illumination system

The video and illumination systems were assembled by Fuhrman Diversified, Inc. in Seabrook, Texas, USA. The imaging system consisted of a monochrome ultra-high resolution CCD chip compact camera sensitive to infrared and visible light that was contained in a cylindrical waterproof housing (95 x 280 mm). A 6 mm (70 degree diagonal) wide angle manual iris lens was used for all observations. The camera was positioned vertically on a post driven into the reef 1 m above an anemone, which provided approximately a ~0.67 x 0.67 m field of view. Camera power was supplied from shore over a coaxial cable, which also returned the video signal to a laboratory where images were recorded (with time, day and date) on Hi8 or SVHS videotapes. Distance between the camera installation on the reef and the laboratory was about 250 m, although deployments up to 1 km are possible with this technology. Illumination at night was provided by 4 waterproof infrared illuminators, each with 36 LED emitters with a wavelength peak at 880 nm; the 4 arrays were positioned at a 45° angle about 0.3 m from the anemone. A second coaxial cable provided power (24 volt, 12 amp/h) to the illuminators from shore. The infrared illuminators were turned on at dusk (18:00 h) and off at dawn (6:00 h). During daylight hours, ambient light provided ample illumination for video recording, even on overcast days.

### Observations and analyses of behavior in the post-settlement period

Recordings were made of ten different anemones at different locations on the reef during Austral winter months (June-September). The exact times of settlement, patterns and mode of movements of settlers from the water column onto the anemone, interactions with resident conspecifics upon arrival, and visits and attacks by various predators during the days following settlement were determined from the video recordings. Image quality at night under infrared illumination was sufficient to discern settlers of three spot dascyllus (10 mm SL) as they arrived at anemones from the water column as well as to identify (at least to genus) and estimate body size of potential predators who entered the vicinity of focal anemones. Here we define a "visit" to an anemone by a potential predator as any approach within camera range.

The data reported here represent seven 24-h periods of observation of focal anemones. For the purposes of analyses we divided each 24-h daily period into two parts - daytime (6:00-18:00 h) and night-time (18:00-6:00 h). This allowed comparison of behavior of predators during the daytime feeding and night-time resting periods of *D. trimaculatus* that occupied the anemones.

## RESULTS

*In situ* observations of early post-settlement behavior

Observations recorded on the videotapes revealed that settlement of *D. trimaculatus* occurred primarily during the second half of the night; all 15 settlement events happened between midnight and 6:00 h. Settlers appeared to locate anemones by olfaction (see Holbrook and Schmitt, 1997 for a detailed description of settlement behavior). Very young *D. trimaculatus* generally were inactive at night, sheltering in contact with the anemone. Most individuals sheltered among the tentacles on the oral disc of the anemone, although some spent periods of time positioned underneath the oral disc alongside the column. Despite the low level of nocturnal activity, there were some interactions between conspecifics during the night, normally involving chases of smaller new settlers by the larger individuals that had settled days previously. Chases lasted from 2 to 60 sec. During the night the damselfish showed no apparent behavioral response (e.g., hiding, cessation of movement) to visits by potential predators. There was no indication that either visual or olfactory cues were being used by the damselfish at night to detect predators.

After sunrise the three-spot dascyllus commenced feeding in the water column. Even newly-settled individuals that had been on the reef just a few hours exhibited this behavior, although for the first day or so following settlement they tended to stay close to the oral disc, rising only 5-10 cm above the anemone to feed. This distance increased with the body size of the fish. Feeding occurred throughout the day and ceased at dusk. During the day the damselfish visually detected potential predators in the vicinity and upon threat, interrupted feeding and took shelter in the oral disc of the anemone. This was accomplished by rapid swimming to the anemone usually initiated by one or two members of the group, but soon followed by all.

Visits by potential predators of juvenile *D. trimaculatus* occurred frequently throughout the day and night, and numbers of visits by predators in each 12-h time period were similar. On average, there were 30.9 predator visits per anemone per night (range 22-40, N = 216) and 28.9 predator visits per anemone per day (range 22-40, N = 202). The vast majority of the visits were made by piscivorous fish 15-40 cm total length in four families: Lutjanidae, Serranidae, Carangidae and Muraenidae (Table I). Invertebrate predators such as *Octopus* sp. visited much less frequently. Although some groups of predators (Lutjanidae and Serranidae) visited throughout the 24 h period, some visited primarily during daytime (Carangidae) and others only at night (Muraenidae; Table I).

Table I. - Patterns of visitation by various groups of predators to focal anemones. Given are the proportion and number of total visits by each group during daytime (6:00-18:00 h) and night-time (18:00-6:00 h).

	Night		Day	
	Proportion	Number	Proportion	Number
Lutjanidae (snappers)	0.20	44	0.23	46
Serranidae (groupers)	0.74	159	0.55	111
Muraenidae (moray eels)	0.05	12	0.00	0
Carangidae (jacks)	0.01	1	0.21	42
<i>Octopus</i> sp.	0.00	0	0.01	3
Totals		216		202

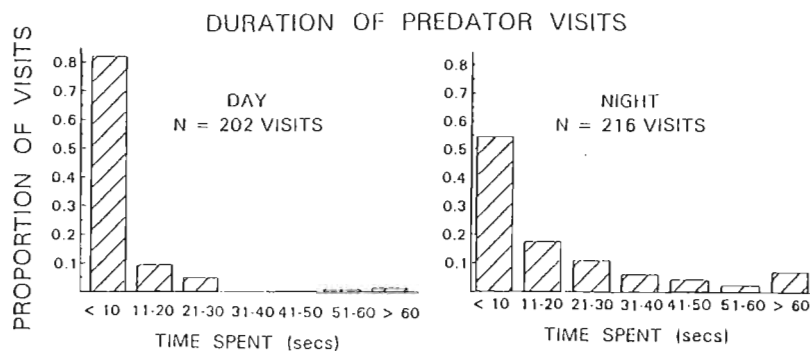


Fig. 2. - Duration of visits by potential predators to focal anemones. Given is the proportion of visits of various lengths during the daytime (6:00-18:00 h; top graph) and the night-time (18:00-6:00 h; bottom graph).

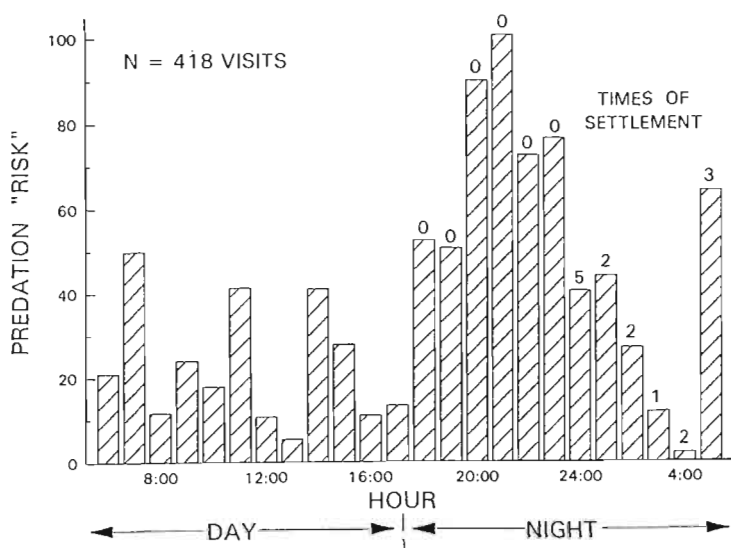


Fig. 3. - Pattern of predation risk to young *Dascyllus trimaculatus* on anemones throughout the daily cycle. Predation risk is the number of visits to a focal anemone per hour made by predators multiplied by the duration of each visit. Values above the bars indicate the number of *D. trimaculatus* that settled during each hour of the night.

The length of time a potential predator spent on a visit ranged from a few seconds to a few minutes. During the day the majority of visits were short - less than 10 s (Fig. 2). At night a higher proportion of the visits were longer, and some lasted several minutes. In many cases the predator displayed little interest, but about half the time (48 percent of night and 45 percent of day visits) the predator actively inspected the anemone. These resulted in 7 attacks. Attacks on damselfish only resulted in capture when the damselfish was not in contact with the anemone, apparently because the predator could obtain

its prey without being stung by the anemone. In one case the predator (a moray eel) made contact with the anemone and reacted in a manner consistent with being stung. Separations of the damselfish from the anemone occurred frequently during the day while the fish were feeding in the water column, and at night following chases by conspecifics. Also, on several occasions the videotapes revealed young fish, seemingly asleep, simply drifting away from the tentacles of the anemone at night.

The temporal pattern of 'risk' from predators (i.e., mean number of visits per hour times mean duration of each visit) was generally low during the day, highest during the first half of the night, lower during the second portion, but high again as dawn approached (Fig. 3). The period of the night where predator activity was lowest was also when the majority of observed settlement events occurred (Fig. 3).

## DISCUSSION

One of the major unresolved issues for demersal reef fishes is the degree to which patterns of local abundance and population dynamics are influenced by presettlement as opposed to postsettlement processes. The resolution of this issue has been greatly hampered by a lack of information about the settlement process and the events immediately following it (Caley *et al.*, 1996; Schmitt and Holbrook, 1996; Holbrook and Schmitt, 1997). Recruitment [the first observation of young on the reef (Keough and Downes, 1982)] usually is assessed several weeks or even months after settlement of reef fishes (Caley *et al.*, 1996), thereby missing the early postsettlement period when mortality rates are likely to be high. Indeed, we estimated that fractional losses of cohorts of three species of *Dascyllus* (including three-spot dascyllus) during the first two weeks after settlement at our study sites in Moorea, FP ranged from 0.31 to 0.55 (Schmitt and Holbrook, 1999). Fractional mortality dropped to between 0.11 and 0.19 for the species during the following two weeks. These high early mortality rates dictate that we obtain more complete information about the magnitude as well as the sources of mortality of reef fishes during the days just following settlement. Only then will we be able to place mortality into context with other processes that affect populations of reef fishes.

The video observations of three-spot *Dascyllus trimaculatus* on Moorea revealed a variety of predators - mostly fishes - frequently visited anemones throughout the diurnal period. Successful attacks by predators occurred only when the damselfish became separated from the anemone. This happened during the day when the fish fed in the water column, or at night when they were chased or moved off the anemone. Although predator visits were as frequent during the day as at night, daytime visits tended to be briefer. The damselfish compensate for the heightened exposure to predation while feeding in the water column during the day by watching for predators and when threatened, by rapidly seeking shelter in the tentacles of the anemone. Living in a group appears to be beneficial during the daytime feeding period because approaching predators can be sighted by any member of the group. At night, predators made longer visits, but the damselfish were protected from predation providing they remained in close contact with the anemone. The only damselfish we have observed captured at night are those that moved away from the anemone, as predators appear to actively avoid contact with the tentacles. Based on the observations of damselfish and their predators, we would predict that different mechanisms could result in changes in predation rates during the night or day. For example, predation rates at night would rise with increasing density of fish on an anemone because



crowding would result in more chasing and displacement of individuals off of the oral disc. During daylight periods density of fish per anemone might be less important to predation risk than ability to visually detect predators. Low light conditions, poor water quality or other factors that impair visual detection could result in increased predation.

*In situ* video observations provide a powerful means to explore the behavior of the young stages of *Dascyllus* as well as their potential predators. Normally, behavioral studies designed to explore early patterns of mortality would be conducted by divers observing fish directly in the reef environment. The remote video system offers several advantages over this approach. First, in the absence of human observers the fish display more natural behaviors. Predation by fishes is rarely observed by divers on reefs due to the inhibitory effects their presence causes on the behavior of the fish, even when viewing conditions are optimal (daytime, high water clarity, etc.). Second, many crucial events such as settlement (Sweatman, 1985b; Robertson *et al.*, 1988; Booth, 1991; Dufour, 1992; Holbrook and Schmitt, 1997) occur mainly at night when direct observations are not possible due to darkness, and introduction of visible light would likely disrupt nocturnal behaviors. The infrared illumination we are using, which is not detected by the fish, enables recording of behavior even under the darkest of conditions. Further, the resolution is sufficiently great that images of small fish, including new settlers, can be discerned. Another great advantage of the video system is that all behaviors are recorded and thus archived on videotape, enabling detailed and repetitive future study without reliance on notes taken in the field by observers.

Despite its advantages in the study of fish behavior, the video system has several shortcomings. Because it is somewhat bulky and is supplied with power from shore, it is most useful for installation at a fixed position on the reef. The visual field at night is quite limited because infrared has a short penetration in water - about one meter or less. Thus the depth of field is not large. This is not a problem in the study of small fishes that have limited movements but clearly would preclude most night-time application to more widely-ranging species.

**Acknowledgements.** - We thank B. Williamson for technical assistance, and W. Holbrook, F. Murphy, K. Seydel, M. Schmitt, S. Larson, and C. Shuman for assistance in the field. This is Contribution 59 of the Gump South Pacific Biological Station. The research was supported by grants from the U.S. National Science Foundation (NSF OCE 91-02191 and OCE 95-03305); funds for infrared video systems were provided by the W.M. Keck Foundation.

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## BEHAVIORAL DETERMINANTS OF THE INSTREAM DISTRIBUTION OF NATIVE HAWAIIAN STREAM FISHES

by

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**ABSTRACT.** - The Hawaiian freshwater ecosystem is comprised mainly of 376 small, torrential mountain streams driven by orographic rainfall. The entire complement of native stream fishes consists of only five species, belonging to the families Gobiidae and Eleotridae, and they share an amphidromous life cycle. Adults live in fresh water where they have a distinct instream linear distribution pattern which varies predictably between streams. Variation in distribution can be explained by the behavior of recruiting juveniles, by the natural ebb and flow of streams in response to rainfall, by stream gradient, especially where the stream enters the ocean, and by the spawning migration in one species. Fish surveys in island streams should not be based solely on the presence or absence of species, but should take into account the behavior of recruiting juveniles in response to stream profile and flow.

**RÉSUMÉ.** - Facteurs comportementaux de la distribution des poissons indigènes des rivières des îles Hawaï.

L'écosystème dulçaquicole des îles Hawaï est constitué de 376 petites rivières de montagne soumises au régime des pluies tropicales. Les poissons indigènes y sont réduits à cinq espèces seulement, appartenant aux familles des Gobiidae et des Eleotridae; ils ont tous un cycle vital amphidromique. Les adultes vivent en eau douce où ils montrent une nette distribution linéaire qui varie entre les rivières. Ces variations dans la distribution peuvent être expliquées par différents facteurs: le comportement de recrutement des jeunes, le régime des rivières en fonction des pluies, le gradient de salinité, notamment dans les cours inférieurs des rivières, et la saison de reproduction. Les études sur les poissons des rivières insulaires ne doivent pas se réduire seulement à noter la présence ou l'absence des espèces, mais elles doivent prendre en compte le comportement de recrutement des jeunes en fonction du profil de la rivière et de son régime.

**Key-words.** - Gobiidae, Eleotridae, Hawaii, Recruitment, Instream distribution.

Unlike the ocean environment, the freshwater ecosystem of Hawai'i has been largely unnoticed and mostly ignored. Information on this small, but critical ecosystem was limited to autecological studies of the native stream animals (see Kinzie, 1989). Statewide stream census of native stream biota was not available to the Hawai'i Division of Aquatic Resources when it was legislatively empowered in 1987 with regulating and managing all surface water. The only available information was from sporadic observations or "one-shot" qualitative censuses for water-use planning or permitting purposes (Hawai'i Stream Assessment, 1990). Other studies on the biota were for purposes other

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than a statewide census, such as the report by Shima (1965-67) who conducted a reconnaissance survey on selected streams suitable for introducing recreational game fishes or the faunal inventory by Timbol and Maciolek (1978) that was limited to perennial streams with modified channels.

The first statewide census of freshwater fishes was by Timbol *et al.* (1980). Their main objective was to obtain definitive information on the statewide distribution and abundance of the gobiid *Lentipes concolor*. Due to fiscal and time constraints, only a single site (a strip 20 m long by 1 m wide) was sampled for selected streams thought to harbor this species. Other information was gathered from the literature or estimated. They reported an alarming absence of many gobies, but especially *L. concolor*, on several islands. Their report prompted the Sierra Club to petition the U.S. Fish & Wildlife Service to list this species as endangered on several islands and threatened on others. However, a more rigorous and thorough census by the State fishery agency (Division of Aquatic Resources), precipitated by this legal process, proved otherwise. The report by Nishimoto and Kuamo'o (1991) contested the report by Timbol *et al.* (1980) and launched a series of long term monitoring projects to understand the natural history of *L. concolor* and other native Hawaiian stream gobies. Our report will explain the role of recruitment behavior in juvenile gobies leading to the linear instream distribution of native Hawaiian stream fishes.

## PHYSICAL SETTING

The Hawaiian Islands, situated in the middle of the Pacific Ocean, is the most isolated archipelago in the world. The high islands in the southeastern section of the island chain range from the northwest at 22°N, 160°W to southeast at 19°N, 155°W. These eight islands, ranging in elevation from 4,000 to nearly 14,000 feet, make up over 96% of the total land mass of the entire Hawaiian chain and contain the only substantial freshwater ecosystem.

The Hawaiian freshwater ecosystem consists of 376 small, torrential mountain streams located mostly on the windward sides of the islands. Home to five species of indigenous fishes, these streams have the following common attributes: 1. They are small by continental standards and have an average annual flow ranging from 253 cubic feet per second (cfs) in the largest gauged stream to as low as 0.21 cfs (Hawai'i Stream Assessment, 1990), 2. The streams are punctuated by numerous waterfalls, which generally gives them a precipitous profile, 3. and they are characterized by flashy flows. Although year-round orographic rainfall is the primary source of stream water, localized heavy rainfall and storms passing through the islands cause frequent flooding.

## NATIVE STREAM FISH FAUNA

The extreme isolation of these islands has resulted in a depauperate fish population coupled with a high degree of endemism (Gosline and Brock, 1960). There are only about 400 species of marine fishes of which about one-third are endemic and only five species of native freshwater fishes (four gobies and one sleeper) with all but one species endemic.

Hawaiian stream fishes share a unique commonality: 1. They represent only two closely related fish families, the Gobiidae and the Eleotridae. Their fused pelvic fins, at

least for the gobies, are particularly adapted to holding on to the rocky substrate even during flash floods. Eleotrids do not have this morphological adaptation, and, consequently, the first waterfall is usually a deterrent to its inland distribution. 2. The fishes have a diadromous life cycle. They lay their eggs in fresh water where the larvae hatch and drift downstream. After a lengthy period of time at sea (up to six months), the young fishes migrate back into the stream mouth as mostly clear postlarvae after their oceanic larval phase. 3. Adults have a distinct instream distribution pattern which varies predictably between streams. *Eleotris sandwicensis*, the only representative of its family, and the goby *Stenogobius hawaiiensis* occupy the lowest stream section. *Awaous guamensis*, *Sicyopterus stimpsoni*, and *L. concolor*, in this order, occupy the rocky, turbulent, and precipitous middle and upper stream reaches. In streams with an estuary or with a gradual profile, all five species show a typical linear distribution pattern with perhaps some overlap. However, for streams that terminate into the ocean as a waterfall the only species may be *L. concolor* and *S. stimpsoni*. The other three species are either absent or are found in the plunge pool near at the base of the waterfall.

### RECRUITMENT BEHAVIOR

The postlarvae of all five species of gobies enter streams year round but are most abundant during periods of freshet or flood flows (Nishimoto and Kuamo'o, 1997; Tate, 1997). They move into the estuary as clear postlarvae, ranging in size from 13.5 to 23.6 mm SL (Nishimoto and Kuamo'o, 1997).

Most postlarvae are clear when transported into the estuary by the rising tide. Those that are slightly pigmented are capable of swimming in. Once in the estuary, the postlarvae quickly become pigmented and move inland by "rock-hopping" on the bottom where it is shallow and swim, usually in schools, when traversing the deeper estuarine sections (Tate, 1997). Juveniles of the euryhaline Hawaiian flagtail, *Kuhlia sandwicensis*, occur in numerous small aggregations in the estuary where they pick off the swimming postlarvae. The postlarvae avoid these attacks by diving to the bottom. When on the bottom, however, they are often ambushed by both the adults and juvenile stages of the native *E. sandwicensis*. These predators, like all stream fishes, are inactive at night and are usually sequestered under large boulders or in side pools. At sunrise, they again appear in the estuary and prey on recruiting goby postlarvae.

The time of recruitment varies by species. *Lentipes concolor* and *Sicyopterus stimpsoni* recruit mostly around sunrise. *L. concolor* move through the estuary singly or in small groups, preferring the stream edges where there is a slight counter-current flow upstream. The postlarvae of this species have large pectoral fins and a highly muscular pelvic sucker, both of which are used to climb waterfalls and maintain position in turbulent flows. This species is an extraordinary climber, using the "powerburst" technique (Tate, 1997) by rowing its pectorals vigorously while detaching the sucking disk from the substrate (Nishimoto, personal observation). Adult male *L. concolor* are highly territorial and have strong site fidelity (Fitzsimons and Nishimoto, 1990). Intruding males, but especially migrating conspecific juveniles, are immediately chased out of the territory. Recruiting juveniles frequently take on a subdued color, similar to an adult female, when circumventing a male's territory during upstream migration.

In contrast, *S. stimpsoni* schools while traversing the estuary (Tate, 1997). Unlike *L. concolor*, juveniles will set up territories as they enter fresh water. They develop

black-bordered orange longitudinal bands on the dorsal fins. This color pattern is apparent during fin-flicks when repelling intruders. During this period, *S. stimpsoni* undergoes rapid metamorphosis of its cranial structure, where the upper lip greatly enlarges and the mouth position shifts from terminal to nearly ventral (Schoenfuss *et al.*, 1997). This is an adaptation for feeding and climbing waterfalls. Juveniles without this change, when tested in a test waterfall apparatus, were not able to climb the vertical face. This species climb waterfalls "inch-worm fashion" with alternative use of its mouth and ventral sucker (Nishimoto, pers. obs.). Other gobies in this genus are well known as rock-climbers (Fukui, 1977). Adult *S. stimpsoni* set up feeding territories, easily recognized by a distinct clear patch cleaned by constant grazing by this herbivore. Courtship also occurs in this area (Fitzsimons *et al.*, 1993). All intruders, but especially conspecifics, are vigorously chased from this patch.

A combination of active swimming through the estuary at sunrise during predator avoidance and an ability to climb waterfalls permit both of these species to move the furthest inland. Intense predation pressure in the estuary displaces young fish inland (Tate, 1997). Similarly, the reduced intraspecific aggression along with ontogenetic development decreases the drive to migrate further and consequently defines its upper instream limits.

The postlarvae of *Awaous guamensis*, the other true rainforest goby, are transported into the estuary throughout the day and night. This species also schools while traversing the estuary. Like *S. stimpsoni*, *A. guamensis* sets up feeding territories upon reaching fresh water. Territory holders have a distinct, black-bordered large orange spot on the dorsal fin, which is displayed during fin-flick behavior. This species spends about four weeks in this area of the stream (Font and Tate, 1994), but the cause of the stay in the estuary by the species is still unclear. The poor climbing ability of this goby (Nishimoto, pers. obs.) and its extended stop at the top of the estuary, limits its inland migration to the middle sections of streams. *A. guamensis* is not aggressive in its adult habitat, except when it mass-migrates downstream after the first autumnal freshet (Ego, 1956; Kido and Heacock, 1991), which signals the onset of the rainy season. Breeding territories are quickly established and strongly defended (Fitzsimons and Nishimoto, 1990). The fate of adults after breeding is still unknown. There is anecdotal observation that all reproductive pairs die after reproducing while others believe that some adults return upstream to the adult habitat. Regardless, the seasonal downstream reproductive migration of this species opens up available midstream habitat for recruiting juveniles.

Postlarvae of *Stenogobius hawaiiensis* and *Eleotris sandwicensis* are nocturnal recruiters. They are poor swimmers in the estuary. *S. hawaiiensis* has a poorly muscularized sucking disk and consequently is unable to climb waterfalls. Unlike the other Hawaiian stream gobies, this species prefers the quiet side pools with a sand or sediment substrate near the stream mouth. *E. sandwicensis* is abundant in the estuary during postlarval migration, however, it prefers the lower stream section, up to the base of the first waterfall. Upon entering the estuary, *E. sandwicensis* immediately takes up residence and starts preying on recruiting fishes. Juvenile coloration is quite distinct from the adult color pattern. The fish are brown dorsally with the sides jet black; the coloration matches the substrate and probably affords protection from predation by the swarming *Kuhlia sandwicensis*.

## INSTREAM DISTRIBUTION OF NATIVE GOBIES

The 1980 study by Timbol and others was limited to single sites in selected streams throughout the State. Because of their sampling gear (heavy electroshocker), their sample sites were limited to areas accessible by automobile. Their "spot-census" method did not account for the distinct linear distribution of native Hawaiian gobies, and their report, if remaining unchallenged, could have resulted in damaging misinformation about the life history of native Hawaiian stream fishes, particularly in reference to the status of *L. concolor*. Our follow-up statewide survey involved in-situ sampling, using snorkel, mask and frequently a neoprene wet suit. Entire stream lengths were covered from the estuary to headwaters. Many Hawaiian streams are in rugged areas and consequently access was frequently difficult. For these reasons, we used a combination of hiking, rappelling, camping, and even insertion into very remote sites by helicopter.

Surveys of island streams should not be solely based on species presence or absence at limited sampling sites, but should consider relationship of recruitment behavior, predator avoidance, climbing behavior to stream profile, and the natural ebb and flow of streams. This approach, we believe, provided us with an accurate assessment of native stream goby populations and a strong foundation for understanding the life history of these fishes and for formulating protective and management measures.

**Acknowledgements.** - The ideas presented in this paper are the result of a series of joint stream-study projects between the State Department of Land & Natural Resources, Division of Aquatic Resources and the Louisiana State University Museum of Natural Science. These studies would not have occurred without the leadership of Mr. Bill Devick, Acting Administrator, Division of Aquatic Resources and the capable field assistance of Mr. Darrell G. K. Kuamo'o.

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## THE UV VISUAL WORLD OF FISHES

by

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**ABSTRACT.** - The workshop on UV vision in fishes brought together a diverse group of researchers to span research areas from molecular genetics to field ethology and animal groups from marine invertebrates to birds. Diverse research areas were required to produce a collaborative understanding of the proximate mechanisms and adaptive functions of UV vision. Without knowledge of the proximate causes, we have little hope of understanding the nature and function of UV vision in fishes. Diversity of taxonomic interest was demanded by the sparse nature of our knowledge of UV vision beyond that for terrestrial insects. The overall conclusion of the workshop is that we stand on the brink of a series of discoveries that will, through knowledge of the functions of UV vision, change our perception of the use of vision by many marine animals.

**RÉSUMÉ.** - Le monde visuel UV des poissons.

L'atelier sur la vision UV des poissons a rassemblé un groupe de chercheurs travaillant sur des sujets différents mais complémentaires. Le but était de mettre en commun les résultats obtenus par des études moléculaires et éthologiques sur divers organismes allant des invertébrés marins aux oiseaux. Ainsi la compréhension collective des fonctions adaptatives et des mécanismes communs liés à la vision UV permet de déterminer les causes des ressemblances de cette fonction chez tous les organismes pour finir par comprendre cette fonction chez les poissons. En effet, jusqu'à présent la connaissance de la vision UV était limitée aux insectes. La conclusion générale de cet atelier est qu'il nous reste encore beaucoup à apprendre sur les fonctions liées à la vision UV et que notre perception de la vision UV des organismes marins changera sûrement.

**Key-words.** - Pisces, Fish, Billfish, Larvae, Aves, Birds, Fish communication, Fish signaling, UV vision.

The text that follows is excerpted from the final reports submitted to the United States Office of Naval Research that provided support for the workshop.

### A NARRATIVE SUMMARY OF THE PAPERS PRESENTED

#### **George Losey: The UV visual world of fishes**

Dr. Losey opened the workshop with a paper that considered the perceptual world of fishes, including the pivotal role of color and color vision in fish communication and signaling, the potential for misleading signals ("lies"), and the question of the reliability of color in the marine environment. He then noted that recent work on ultraviolet vision in fishes shows that the significance of this spectral region in their sensory worlds has

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been completely overlooked. This workshop is intended to address this deficiency in order to understand the significance of UV in the biology of particular fish species, and to predict the potential effects of a worldwide increase in solar UV irradiation due to ozone loss. Specific, narrower goals are as follows:

- (1) Share knowledge among workers in the field.
- (2) Identify major research questions.
- (3) Discuss the most appropriate technical approaches.
- (4) Form new cooperative research efforts.
- (5) Identify possible sources for funding.

George finished his presentation with a description of the new University of Hawaii UV camera designed for underwater use, and expected to be in service by mid-1998.

#### **William McFarland: Ultraviolet radiation in tropical seas**

Dr. McFarland noted the indigo color of the sea, which suggests that very short wavelengths are well transmitted into (and scattered back out of) marine waters. He defined the UV-A, UV-B and UV-C regions of the spectrum, and further defined natural UV light as the portion of the measurable spectrum between 300 and 420 nm (the upper limit being set by the absorption of a 360 nm visual pigment). By this definition, horizontal light in the sea contains up to 38% of its total photons in the UV range, and even in relatively less clear coastal waters, nearly 30% of the photons are in the UV off Catalina Island, and these make up 17% of total photons even in the quite murky waters of Kaneohe Bay.

McFarland made two other significant observations relating to the potential importance of UV light in the sea: (1) Assuming a reasonable threshold, down-welling UV light in the open sea is visible at a depth of at least 280 m during the day (~230 m looking horizontally), and (2) the proportion of UV light in the spectrum is actually **greatest** at night, due to celestial scattering.

#### **Timothy H. Goldsmith: Measuring and understanding visual sensitivity of vertebrates to ultraviolet light**

Professor Goldsmith noted that scientists have been aware of the presence of UV visual photosensitivity in vertebrates for only the last 25 years or so. He pointed out that while all visual pigments have some absorption in the UV, there are specialized UV photoreceptors that absorb only in this spectral region. Technically, a standard approach has been to use microspectrophotometry (MSP), which is a relatively insensitive technique away from the lambda-max. Using a suction electrode technique, Goldsmith's lab has been successful in characterizing the UV rhodopsin of the zebrafish *Danio rerio* down to 4 logunits below the maximum.

Tim next pointed out 3 special characteristics of UV photoreceptors: (1) They have narrower spectral band widths than those of receptors peaking in the "visible" region of the spectrum, (2) they have a wavelength of maximum absorption that is shorter than that of the retinal chromophore, a wavelength shift that is produced by an unknown mechanism, and (3) they often have sensitivity at wavelengths well above that of the UV peak, suggesting the presence of additional visual pigments in a single photoreceptor.

The presentation then discussed the differences between color vision and other spectral modalities, such as wavelength-specific behaviors, or multiple behaviors that have different involvement of spectral mechanisms. This led naturally to a discussion of recent work with birds (budgerigars: Psittacidae) in Goldsmith's laboratory, where the presence of true UV color vision has been documented as indicated by 3 results. First, the

budgies can tell pure 440 nm light from 440 nm light + 10% UV. Second, the budgies can discriminate 440 nm from 420 nm light at all intensities. Finally, a 420 nm light can be matched by a mixture of UV + 440 nm light, forming a metameric match. This suggests that 2 receptor classes, a UV-sensitive and a violet-sensitive type, interact to discriminate color in the short blue and UV region of the spectrum. The longer wavelength receptor classes are apparently isolated by virtue of the highly absorptive pigments in their cone oil droplets.

### **Thomas W. Cronin: Ultraviolet vision in marine invertebrates**

Dr. Cronin began by amplifying McFarland's earlier remarks about the great penetration of UV light into clear oceanic waters, and discussed the presence of short-blue photosensitivity (extending into the ultraviolet) in midwater oplophorid shrimps. In these animals, the short-wavelength photoreceptors interact with middle-wavelength receptors in such a way that discrimination between down-welling light and bioluminescence is optimized.

Cronin showed a series of photographs of coral reef scenes showing how UV imaging compares with imaging in the blue, green, and red regions of the spectrum. Contrast at short range is much higher in the UV, due to silhouetting of fish and other objects against bright UV "spacelight". This suggests that UV vision might be most useful at short visual ranges.

The presentation continued with a discussion of the multiple UV channels present in retinas of mantis shrimps. These shallow-water marine invertebrates have several UV receptor classes, of which some are also polarization sensitive.

Tom concluded by listing several potential functions for UV vision: (1) recognition of species-specific bioluminescence; (2) recognition of species-specific UV color patterns; (3) navigation and orientation using UV polarization patterns; (4) analysis and imaging of polarized UV reflectance patterns; (5) signaling in the UV; (6) breaking of UV camouflage; (7) imaging midwater objects against the bright UV underwater spacelight; (8) avoidance of excessive UV photoexposure.

### **Justin Marshall: Colours and their function in reef fish**

Dr. Marshall began his presentation by discussing two useful items of field instrumentation, the famous submersible instrument known as SubSpec, which is capable of making full-spectrum (300-850 nm) measurements of spectral irradiance and reflectance either in the field or in the lab, and a submersible UV video system based on a commercial handycam plus appropriate filters. (The latter system was featured in a video shown later in the day, in which fish visible colors were compared with their appearances in the UV.)

The talk then considered the complex spectral reflectance of fishes, illustrating both simple and complex colors. Justin pointed out that fishes and birds have hit upon similar color signaling systems, in which adjacent colors reflect in mutually different spectral regions. This would presumably provide a strong color contrast when seen by the appropriate visual system. In fact, with regard to the UV, there can even be pairing of fluorescent and non-fluorescent colors (as on the face of the budgerigar - *Psittacidae*).

Several models of UV and visible color vision were presented. The talk concluded with two observations. First, UV is likely to be a short-range signaling system underwater, and second, that other spectral regions (in particular the infrared also visible to many animals and should be considered in any study of color and signaling).

**David Hyde: Cloning and molecular characterization of the zebrafish UV opsin**

Dr. Hyde is particularly interested in using zebrafish, *Danio rerio*, as a developmental system for the study of visual function and degeneration in humans. He has recently cloned all of the visual pigments of zebrafish. The retina contains 4 types of photoreceptors: rods, double cones (with 2 members each), long single cones, and short single cones. Using PCR, 6 opsin classes were found to exist in the retina, which correspond taxonomically (in order of decreasing lambda-max) to a red class, 2 different green classes, a rod class, a blue class, and a UV class. The UV class is quite different from the UV pigments of invertebrates, but is similar to the UV opsin of goldfish, *Carassius auratus*, another Cyprinidae.

David's laboratory developed an elegant way to produce antibodies against each of the visual pigment classes with little or no cross-reactivity between classes (except for the very similar green types). Using this method with retinal slices, he showed that the double cones contain the red and green pigments, the long single cones contain the blue pigment, and the UV pigment resides in the short single cones.

**Kerstin Fritches: Vision in billfish (Istiophoridae)**

Billfishes (marlins) form an interesting group of fishes, and have a number of unusual visual adaptations including huge eyes and the presence of a heater organ (formed from the *superior rectus* extraocular muscle) near the retina. Ms. Fritches has isolated retinas from these animals and determined the local concentrations of ganglion cells in various retinal regions (which is a measure of the distribution of visual acuity throughout the retina). Marlins have their best vision in the nasal and temporal retinal regions (looking ahead and to the side), but their best resolution seems no higher than 9 cycles per degree, which is fairly low. This shows considerable convergence of cones onto ganglion cells.

The lenses of billfishes absorb strongly in the UV (cutting off at wavelengths below 400 nm), making it very unlikely that there is residual UV photosensitivity at the level of the retina. However, the animals may well have color vision since the retina contains both single and double cones in a square mosaic in the ventral retina (the dorsal retina has only a row mosaic of double cones).

Kerstin showed that marlin actually have strong, rapidly changing color patterns visible in the UV, which may be important in their interactions with prey. This aspect of their photobiology is to be studied more thoroughly in the near future using a lure camera to videotape body coloration changes during strike behavior.

**Andrew Bennett: The function of UV vision in birds**

Potential uses of UV vision are for crypsis, warning, sexual selection, and mimicry. Other aspects of UV photosensitivity may include setting circadian rhythms or playing a role in navigation and orientation. Dr. Bennett's talk provided an opportunity to consider some cases where UV vision is well studied, and its significance is well established. He has worked with mate selection in two species of birds, the zebra finch and the European starling. These animals both reflect UV light in particular patterns and have visual receptors specific to the UV region of the spectrum.

Bennett's work with the zebra finch showed that the female finches preferred males when they could observe both their UV and visible color patterns, and furthermore that they preferred males with symmetrical UV colored markings. Female starlings, on the

other hand, chose males in both UV+ and UV- conditions, but they made different (and reproducible) choices in these two cases. When using UV colors in their choices, they preferred males with particular UV reflectance spectra. Thus, in both these bird species, the UV appearance of a male has a major effect on its attractiveness to females.

#### **William McFarland: Larval fish feeding in the ultraviolet**

In the final paper in the workshop, Dr. McFarland presented data showing that larval stages of the grunion and the top-smelt (Atherinidae) feed effectively in environments containing only ultraviolet wavelengths of light. These larval fishes have 3 known visual pigments in their cones: a pair of double cone pigments (maxima at 441 and 535 nm) and one single-cone pigment (maximum at 355 nm).

Larval fishes were fed in UV light either produced by a monochromator or by passing sunlight through a Hoya UV-transmitting filter. In both cases, capture of prey (*Artemia salina* nauplii) was rapid and successful. In the dark, however, few or no prey were taken by the larval fish. This is strong evidence that the fishes are capable of imaging prey in the UV alone.

Plankton are known both to reflect UV light and to stand out in contrast against a UV background, so this may explain why UV vision is particularly useful to fish larvae. Mac noted that there is plenty of UV light in the shallow marine environments in which these larvae naturally occur.

### **GENERAL DISCUSSION**

Discussion centered on directions for future research. Three areas of consensus were reached:

1. There should be two thorough research surveys carried out, one to examine how widespread is the occurrence of UV vision in various taxa and ecological classes of fishes, and the other to examine the specific role of UV vision in behavior of target fish species in the field and in the lab.

2. The cost of UV vision should be considered, particularly with regard to retinal damage or possibly to visual acuity compromise by chromatic aberration in larger eyes. The receptive field structure of UV vision systems should be examined as well, using classical electrophysiological approaches.

3. The workshop should generate a paper which will act as a UV vision primer for fish biologists (and for other field biologists interested in animal ecology and behavior). The level of ignorance concerning UV coloration, and the different perceptual worlds of animals compared to humans, makes such a document highly desirable. Workshop participants agreed in principle to produce such a document.

One of the most basic questions addressed in discussion sessions was whether research on UV vision deserved special, concentrated attention. Is this merely an extension of vision into slightly shorter wavelengths, a "deeper indigo blue", that is likely little different from blue-sensitive vision? Three compelling reasons for concentrating on UV vision were identified:

1. UV, at the low end of the spectrum, has special properties in sea-water. Light at the long end of the spectrum is largely absorbed but short wavelengths are strongly scattered. When compared with blue to red spectra, this scattering results in qualitative differences in the UV-perceptual world. Clear oceanic water appears as a bright UV background

against which close, blue-camouflaged objects stand out in strong contrast. Fine UV reflectance patterns may be distinct in a close object but blur to complete loss of detail at long distances.

2. Hypotheses concerning the behavior, ecology and evolution of fishes have been based largely on human visual perception and thus cover only the visible spectrum from about 400 to 780 nm. A concentrated effort is required to extend this knowledge into the UV to include the full range of the sensory world of fishes.

3. UV-sensitive cone cells in the retina of the goldfish have some special properties that are not found in other visual receptors and may reflect special functionality.

It was agreed that, at this early stage in research, broad survey work is still required. Comparison of the use of UV reflectance by species should be made across broad ecological guilds in pursuit of ecological or behavioral factors that correlate with certain types of UV coloration. Quantitative study of spectral reflectance of fish pigments is beginning to reveal "families" of pigments such as "yellow" vs. "UV-yellow" and the species that use these pigments (N.J. Marshall). Enlarging this survey to include diverse species will enable comparative analysis of the possible functions of the UV reflectance. The UV video system being developed by G. Losey will be a key element in this survey by allowing us to visualize the dynamic visual world of fishes and quickly switch between various visual spectra and angles of polarization. Use of a 1:1 gamma correction in the camera will enable rough quantification of spectral luminance and contrast within the fishes' coloration and between the fish and its background. Knowledge of which species have UV reflectance that differs from the visible spectrum will aid workers who lack UV visualization equipment.

It was also agreed that, along with the broad-scale survey, detailed physiological and experimental work should be emphasized. The most basic level is the study of the eye itself. At the most crude level, estimation of the proportion of UV light passed through the eye to the retina can be used as an adjunct to the field surveys to indicate which species likely have UV vision. However, ERG or MSP studies are required to verify the presence of UV-sensitive cones in the retina. The more recently developed molecular techniques to detect the presence of various types of visual pigments have been validated only for the zebra fish at present. However, the apparently conservative nature of the genetic basis for visual pigments suggests that this could become a fast and accurate method if it can be validated across species.

Also at the level of the eye is determination of the adaptive costs of UV vision. On the one hand, increase of the breadth of wavelengths that are passed to the retina will increase the degree of chromatic aberration induced in the formed image. This is likely a cost, especially for large-eyed species. More controversial is the damage that may be caused to the eye by passing UV-A radiation through the cornea and lens. Some workers have assumed that this must levy a cost but the evidence is largely circumstantial. We need study of structural changes in the lens and retina and damage to retinal cells and photopigments following exposure to physiological doses of UV-A.

Finally it was agreed that, even though our knowledge of UV vision in fishes is in its early stages, detailed experimental study of its functions should begin now. Various evidence points to detection of pelagic objects as the primordial function of UV vision in fishes. Recent underwater photos by T. Cronin are strongly suggestive of a camouflage-breaking function for UV vision. Experiments by W. McFarland and collaborators have indicated that plankton feeding can be accomplished with UV light alone in some species.

This work should be extended to other species from various habitats and expanded to compare the efficiency of feeding in a normal spectral regime with and without UV light.

Regardless of the primordial function of UV vision, once the sensory capability was present it would doubtless have been evolutionarily co-opted for other functions. The social importance of UV reflectance has been indicated by displays in birds and reptiles that employ UV reflective pigments. A. Bennet and others have indicated its importance in mate choice decisions and sexual selection. Experiments should begin immediately to examine social and symbiotic responses of fishes such as mate choice in UV rich vs. UV poor conditions. Work on the zebra finch also warns us that we should not restrict our efforts to species that have striking UV reflectance patterns. Even subtle UV patterns should be examined in species identified as possessing UV vision.

Our best hope of understanding the functions of UV vision may lie in comparing species within a group such as the damselfish (Pomacentridae) that likely had UV vision early in the evolution of the group but now exist in diverse ecological and behavioral states. Differences in the selective regimes that have been faced by these species will likely have led to different life history strategies in the use or loss of UV vision. Even in species that lack UV vision, selection pressure from predators, parasites and mutualists that do have UV vision may have produced special adaptations in terms of UV reflectance patterns.

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## Workshop 7

### FROM FIELD TO LABORATORY: ELASMOBRANCHS AS CONTEMPORARY BIOLOGICAL MODELS

Chairperson: William HAMLETT

HAMLETT W. - From field to laboratory: Elasmobranchs as models in contemporary biology. 829-840

*Du terrain au laboratoire: les élasmobranches comme modèles en biologie contemporaine.*

WALLER G.N.H. - Magnetic resonance imaging study of the holotype of *Centroscyrnus macracanthus* Regan, 1906 (Squaliformes: Somniosidae). 841-845

*Étude de l'holotype de Centroscyrnus macracanthus Regan, 1906 (Squaliformes, Somniosidae) en imagerie par résonnance magnétique.*



## FROM FIELD TO LABORATORY: ELASMOBRANCHS AS MODELS IN CONTEMPORARY BIOLOGY

by

William C. HAMLETT (1)

**ABSTRACT.** - This workshop presented an overview of the past and present themes of research being pursued on elasmobranchs in the world, with particular reviews of current research in Australia, Japan, USA, France and Brazil, as well as with some emphasis on new investigation methods, shark management and role of non-governmental organizations in shark conservation.

**RÉSUMÉ.** - Du terrain au laboratoire: les élasmobranches comme modèles en biologie contemporaine.

Cet atelier a présenté un panorama des recherches passées et actuelles menées sur les élasmobranches dans le monde. Des points particuliers ont été développés: une revue des recherches actuelles menées en Australie, au Japon, aux USA, en France et au Brésil; la présentation de nouvelles techniques d'étude; la nécessité de gérer les populations de requins; enfin le rôle des organisations non-gouvernementales dans la conservation de ces populations.

**Key-words.** - Elasmobranchii, Systematics, Biology.

Traditionally, studies on elasmobranchs have focused on aspects of field biology including abundance, distribution, systematics, age and growth, behavior and potential attacks on humans. These studies continue to be useful, particularly in light of the increased awareness of the deleterious effects on shark populations by overzealous commercial and recreational fishing and bycatch mortalities. Increasingly, laboratory based scientists are appreciating elasmobranchs as attractive models in which to investigate and elucidate many fundamental vertebrate processes. This workshop was designed to present an overview of the present themes of research being pursued on elasmobranchs with an emphasis on the considerable diversity of these areas, in particular laboratory based projects.

Elasmobranchs are often considered primitive, and this is true of many of their structural characteristics, many of which have been conserved with little change. In other respects, they demonstrate characteristics that are "advanced". They have large brains, a well developed nervous system, internal fertilization, viviparity, an endocrine regulatory system similar to higher vertebrates and physiological processes and mechanisms common to vertebrates in general.

Elasmobranchs are a currently under exploited model system that may be effectively employed to investigate and elucidate many important biological and evolutionary processes. Apart from their importance as the basic vertebrate used in comparative anatomy classes throughout the world, they have special relevance as laboratory animals.

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Many mechanisms common to all vertebrates are present in elasmobranchs in an elegantly simple model, unencumbered by adaptations to land and homeothermy. There are also many specialized mechanisms unique to them. Elasmobranchs come in tremendously varied sizes, shapes, differing reproductive modes and specialized physiological mechanisms unique to their environmental niche. An investigator may draw upon such species specializations layered on the fundamental vertebrate body plan to select an appropriate model in which to investigate both basic and unique hypotheses. Numerous important questions remain to be answered about the basic biology of this group, and it is vitally important to stimulate continued research in this direction as well. Increasingly, elasmobranchs are being utilized as valid experimental models with applications in biomedical research. The results of such research may inevitably lead to practical applications in clinical medicine.

Aristotle made the first significant observations on the natural history of Elasmobranchs (Cole, 1949). He knew that some sharks were oviparous and that others were viviparous with a placenta. Hieronymus Fabricius (Adelmann, 1967) reviewed Aristotle's observations and added his own. He drew the ovary, uterus and embryos in *Galeus laevis*. Belon (1551) knew of the attachment of the embryo to the maternal oviduct by means of a "navel string" and Rondelet (1554) depicted such a connection in *Mustelus laevis*. Steno, the Danish anatomist (1673), claimed the connection was a functional placenta. Stefano Lorenzini produced a monograph on *Torpedo* in 1678 and made the first mention of red and white muscle. Malpighii made observations on fish reproduction including observations on the shark placenta (Adelmann, 1966). Müller (1842) presented details of the development and organization of the placenta. Balfour (1878) concentrated on embryological studies and Ziegler and Ziegler (1892) and Ruckert (1899) were concerned with early development. Various others produced ichthyological volumes including information on elasmobranchs including Beard (1890), Cuvier and Valenciennes (1828-1845) and Dean (1909). The work of Garman (1913) was an important contribution and has recently been reprinted and is available to a new generation of students. In 1932 and 1934 Ranzi published important syntheses on reproductive modes in elasmobranchs.

In 1922, J. Frank Daniel published the first edition of the now classic, *The Elasmobranch Fishes*. The work subsequently went through three editions. It represented the ultimate compilation of information on elasmobranch morphology at its time. Few other volumes have been so consistently appreciated by students in the field.

Various compilations of individual papers, some from symposia and workshops on elasmobranchs have appeared in the literature. In 1963, Gilbert published *Sharks and Survival* and in 1967 *Sharks, Skates, and Rays*. In 1970, Lineweaver and Backus published *The Natural History of Sharks* followed by Budker's *The Life of Sharks* in 1971 (published first in French in 1947). Both of these volumes were well received by the general public. In 1976, Thorson edited a volume on the ichthyofauna of Lake Nicaragua including much of his own work on the bull shark, *Carcharhinus leucas* and the sawfish, *Pristis perotteti*. In 1976, a symposium on shark biology was held in New Orleans and the results were subsequently published in the *American Zoologist* in 1977. A volume dedicated to sensory biology of elasmobranchs appeared in 1978 (Hodgson and Mathewson, 1978) and Compagno published the much referenced *Sharks of the World* in 1984. Elasmobranchs as models in human biology were the topic of a symposium in Bruxelles, Belgium in 1986. The results appeared in the *Archives of Biology (Bruxelles)* (Hamlett, 1987). In 1988, two important volumes appeared, Compagno's *Sharks of the Order Carcharhiniformes* and Shuttleworth's *Physiology of Elasmobranch Fishes*. A symposium

held in Rome, Italy in 1988 entitled "Evolutionary and Contemporary Biology of Elasmobranchs", brought together the leading workers on elasmobranch biology. The twenty papers presented at the symposium was published the following year in the *Journal of Experimental Zoology* (Hamlett and Tota, 1988). Three symposia in the early 1990's were eventually published as *Elasmobranchs as Living Resources* (Pratt, Gruber and Taniuchi, 1990), *Vision in Elasmobranchs* (Hueter and Cohen, 1991) and *The Reproduction and Development of Sharks, Skates, Rays and Ratfishes* (Demski and Wourms, 1993). In 1996, Klimley and Ainley edited a book on the biology of the great white shark, *Carcharodon carcharias*. An extensive volume of current reviews on elasmobranch anatomy, ultrastructure, biochemistry and physiology using a systems approach will appear as *Biology of Elasmobranch Fishes* in 1999 with Hamlett as editor. This volume will serve as an introduction to the biology, anatomy, physiology and biochemistry of elasmobranchs as well as a reference resource to the primary literature based on contemporary laboratory studies.

Often access to animals and biological materials, in particular elasmobranchs is an impediment unless the investigator is located at a coastal institution. Laboratory based studies at other institutions must rely on limited commercial sources and collaborations. Commercial sources are few and deal only in a limited number of species. If an investigator is interested in a particular topic, such as reproduction, it is often impossible to obtain the desired stages in gestation from commercial sources. Fortunately, many field based elasmobranch programs welcome collaboration from investigators interested in cell and tissue biology, which is a benefit to all concerned.

Elasmobranchs represent a relatively untapped resource outside the traditional range of conventional species as a source of models. Because of homologies among vertebrates, data from non-mammalian systems can help to further knowledge of evolution and physiological control in mammals as well as reveal basic principles. Study of non-mammalian vertebrates should be regarded as having the same application to research in biomedicine as work on species more phylogenetically related to humans.

Unique, unconventional model organisms frequently possess some morphological or functional feature or adaptation to a particular environment that makes them especially well suited for study of a specific character. This is certainly true of elasmobranchs.

Access to the extant literature on elasmobranchs is often difficult to obtain. Much work is done at research centers and universities outside English speaking countries and these papers frequently appear in journals from their home universities with limited worldwide distribution. In addition many of these journals are not referenced in such data bases as Current Contents. Many of the older works were initially published in obscure journals.

A recent search of Biological Abstracts for 1995 and 1996 listed only 96 titles under Elasmobranch. Certainly many more publications were produced but failed to be listed in this source. A search for the same years generated 75 titles for *Squalus*. As a researcher located at a medical school, I have limited access to much of the fisheries literature but abundant access to the biomedical literature. MedLine is an excellent source of information on biomedical topics but does not survey many strictly biological journals. Current References in Fish Research is a private endeavor that generates a list of generally over 4,000 articles yearly in over 400 journals. Even when this source is consulted many omissions occur, in this case many of the medical based topics are omitted. In a survey of this source for the years 1991, 1993 and 1995, generally few references appeared for most elasmobranchs species. Not surprisingly, the vast majority of references were for *Raja*,

*Torpedo* and *Squalus*. This is a valuable resource but points out that researchers interested in certain aspects of elasmobranch biology must search long and deep from a broad based literature pertaining to their topics of interest and the specific species of interest. *Raja*, *Torpedo* and *Squalus* are of particular value in that they have a large extant literature, are available commercially and are of adequate size for use as a laboratory animal.

Most researchers choose the journal for the transmission of the results of their work based on the area of research, not the group of animals involved. For example, a cell biologist dealing with electron microscopy of elasmobranch tissues might choose *Cell* and *Tissue Research* over the *Journal of Fish Biology*. Both are excellent for their particular audiences, but underscores the difficulty in keeping abreast of the elasmobranch literature.

Few publications and newsletters are devoted exclusively to elasmobranchs. Of note are *Shark News*, *Chondros*, *Shark Tagger*, and the NOAA Technical reports series (which frequently features elasmobranch symposia). The Mt. Desert Island Biological Laboratory primarily hosts researchers devoted to elasmobranch studies and the *Bulletin of the Mt. Desert Island Biological Laboratory* publishes reports of their work.

The availability of the Internet is proving of use. A recent search for "Elasmobranchs" using a popular search engine brought up 487 hits. Such bulletin boards as *Elasmo-L* and *Shark-L* are also useful and popular.

In recent years several organizations have been established focused exclusively on elasmobranchs. Most have a robust membership, newsletters and web sites. The American Elasmobranch Association and the Japan Elasmobranch Society are well established with several hundred members each. The Brazilian Elasmobranch Society (*Sociedade Brasileiro para o Estudo de Elasmobranquios*) was established in 1997. Brazil also has an active working group of scientists for fisheries research on sharks and rays that holds an annual meeting. The European Elasmobranch Association has several share holding members including: *Deutsche Elasmobranchier Gesellschaft* (DEG in Germany), *Gruppo Italiano Ricercatori Sugli Squali* (GRIS in Italy), *Associacao Portuguesa para o Estudo e conservacao de Elasmobranqueros* (APECE in Portugal), a Netherlands Elasmobranch Group, The Shark Trust in the United Kingdom as well as the Irish Elasmobranch Group. The number of member countries will undoubtedly continue to grow. The Pelagic Shark Research Foundation, the Shark Research Centre of the South African Museum, Moss Landing Marine Laboratory and Monterey Bay Aquarium are a few of the other locations actively involved with research and community based projects on elasmobranchs.

#### **FROM FIELD TO LABORATORY: ELASMOBRANCHS AS MODELS IN CONTEMPORARY BIOLOGY**

On Friday, November 7, 1997 at 8:00 AM, the workshop, "From field to laboratory: elasmobranchs as models in contemporary biology", was called to order at the ORSTOM Center in Noumea, New Caledonia in association with the 5th Indo-Pacific Fish Conference. Nine speakers presented updates on various aspects of elasmobranch research worldwide followed by vigorous discussions.

##### **Review of current research on elasmobranchs in Australia**

John Stevens, CSIRO Marine Laboratories, Hobart, Tasmania presented an extensive report on the current status of chondrichthyan research in Australia. The majority of

full-time chondrichthyan research in Australia is currently carried out at government agencies and is related to commercial fisheries for sharks. Research on chondrichthyan systematics at museums continues on an "ad hoc" basis. The number of chondrichthyan projects at universities is increasing but there are few research groups specializing in this group of fishes.

Government agencies in Australia include the Marine and Freshwater Research Institute, Queenscliff, Victoria. Headed by Terry Walker, the Southern Shark Fishery group investigates the biology, ecology and stock assessment of school (*Galeorhinus galeus*) and gummy (*Mustelus antarcticus*) sharks. Other areas of emphasis are stock assessment of school and gummy sharks (AFMA funded); tagging to determine mixing rates (FRDC funded); catch monitoring program (FRDC funded); age and growth (FRDC funded) and gillnet selectivity studies (drop out) (FRDC funded). Some work is directed at bycatch species including sawsharks (*Pristiophorus* spp. and elephant fish *Callorhinchus milii*).

Headed by Colin Simpfendorfer, the Western Australian Marine Research Laboratories, Perth, WA studies the commercial fishery for dusky (*Carcharhinus obscurus*), whiskery (*Furgaleus macki*), and gummy sharks. Biology and stock assessment of Western Australian commercially important shark species is also included (FRDC funded). Projects include age and growth of all commercial species, location of nursery areas for whiskery sharks, tagging of whiskery sharks and biology of dusky sharks. A recent FRDC funded study is stock assessment of commercially important large coastal and demersal sharks, which involved tagging of juvenile dusky sharks to determine exploitation rates in nursery areas.

CSIRO Marine Laboratories, Hobart, Tasmania oversees several diverse projects. John Stevens is involved with taxonomy, systematics and biogeography, Peter Last is spearheading the publication of the FAO guide to batoids of the world and CSIRO published Stevens and Last's *Sharks and Rays of Australia*.

Stock assessment and population modeling of school and gummy sharks in the Southern Shark Fishery are concerns of Andre Punt and Yongshun Xiao, as well as age-structured spatial models.

Barry Bruce heads a group studying biology, ecology, behavior, fisheries and conservation. Other topics of study are nursery areas of school and gummy sharks; archival tagging of school sharks; stock structure of school and gummy sharks; catch monitoring, biology, movements and behavior of white sharks (*Carcharodon carcharias*); and movements and behavior of whale sharks. Dietary studies, as part of slope ecosystem projects, involve Tony Koslow and Nic Bax. Chemistry and marine oils, liver oils of deep-water dogfish and squalene extraction are studied by Peter Nichok.

CSIRO Marine Laboratories, Cleveland, Queensland pursues dietary studies relating to prawn predation with reference to the Northern Prawn Fishery and bycatch in the Northern Prawn Fishery under the direction of Steve Blaber.

The Australian Fisheries Management Authority, Canberra, ACT oversees the Southern Shark Fishery, David Johnson, Manager. The Australian Bureau of Agriculture and Resource Economics, Canberra, ACT studies the economics of the Southern Shark Fishery and the Northern Fisheries Center, Cairns, Queensland centers on shark control as does the Fisheries Research Centre, Cronulla, New South Wales.

CSIRO Division of Tropical Agriculture, JM Randall Laboratory, Rockhampton, Queensland is home to Greg Harper, who has a proposed project dealing with a comparison of anti-cancer properties of shark cartilage with cartilage from other sources (bovine etc).

Several projects are being conducted at various universities. Meri Peach is a Ph.D. student at Sydney University (supervisors at Sydney, David Patterson and Greg Rouse) carrying out an ultrastructural study of the pit organs and neuromasts in the lateral line canal and using electrophysiological techniques to test hypotheses about the function of pit organs and to date have examined *Mustelus antarcticus* and *Carcharias taurus*. She will soon be transferring to the University of Queensland (Brisbane) to pursue electrophysiology studies with Justin Marshall. At the University of Queensland, Mike Bennett's group (Department of Anatomical Sciences) is interested in the biology of reef dwelling sharks *Hemiscyllium ocellatum*, *Carcharhinus melanopterus*, *Negaprion acutidens*, *Chiloscyllium punctatum* and *Orectolobus ornatus*. Population structure, size and movements, growth rates (tagging study); reproductive steroid hormone profiles/reproductive behavior and status; parasitological studies; diet and feeding behaviors; healing responses to tag-wounds. Heavy metal accumulation in demersal chondrichthyans in Moreton Bay (with Darren Paul) is also an area of study of the group. They investigate the tissue levels of heavy metals in specific species of sharks and rays and attempt to link these with diet, behavior, age and locality of the fish. Targeting *Orectolobus ornatus*, *Aptychoterna rosstrata*, *Chiloscyllium punctatum* (and *C. leucas* in the Brisbane river), the project involves investigating many aspects of the general biology of these species to assist in data interpretation (e.g., acoustic telemetry of individuals to assess "home ranges", etc.). The group is also involved in a study of the branchial vascular morphometry and morphology of a range of chondrichthyans including *Hexanchus* and *Notorhynchus* (with ex-honours student Megan Storrie). Michelle Heupel is a MSc student with Mike Bennett investigating the biology and behavior of *Carcharhinus melanopterus* at Heron Island. Gillian Renshaw and Graham Wise in Anatomical Sciences study hypoxia tolerance in *Hemiscyllium ocellatum*. Ian Whittington and Leslie Chisholm study systematics and life-history of chondrichthyan parasites at the University of Queensland and Justin Chidlow is a MSc student of Gary Russ studying the biology of wobbegongs (*Orectolobids*).

The University of New South Wales (Sydney) is home to Ricky Chan, Ph.D. student. Biological studies of sharks caught in NSW waters (supervisors: Pat Dixon and Julian Pepperell). Project description: General biological studies of sharks (Families Lamnidae, Carcharhinidae and Sphyrnidae) caught by NSW protective beach meshing program and recreational game fishers. Studies include species identification through DNA techniques (marine forensics), length-weight relationships, stomach-content analysis and reproductive histology. The research program is run through FRI, Cronulla, by Gary Henry and Dennis Reid. A future component of the project will involve looking at the setting of mesh nets and methods to decrease bycatch and possible implementation of tag and release of sharks without reducing public safety. Klaus Rohde is concerned with systematics and life-history of chondrichthyan parasites at the University of New England (Armada, NSW).

Denise Newbound is a Ph.D. student at the University of Western Australia studying stock structure of whale sharks (*Rhincodon typus*), including use of external parasites. She has recently completed an honors project on osmoregulation and ionoregulation of Port Jackson sharks (*Heterodontus portusjacksoni*).

Several studies are underway at Murdoch University (Perth, Western Australia). Margaret Platell is a student studying habitat and dietary resource partitioning of four urolophids in south-western Australia. She will subsequently, examine the reproductive biology and age and growth of these species. (2 species of *Urolophus* and 2 species of *Trygonoptera*). Brad Norman is a MSc student (supervisors Ian Potter and John Stevens)



working on behavior of whale sharks impacted by the ecotourist industry at Ningaloo reef. Geoff Taylor is a MSc student (supervisor Ian Potter) studying aspects of the biology and ecology of whale sharks at Ningaloo.

Mike Heihaus is a Ph.D. student at Simon Fraser University, Vancouver, BC. His interest is influences of prey availability and predation risk on habitat selection of bottlenose dolphins in Shark Bay, WA and tiger shark (*Galeocерdo cuvier*) tracking.

Janine Caira, University of Connecticut, studies systematics and life-history of chondrichthyan parasites in Australia.

Margaritta Margoles is a Ph.D. student from Barcelona University, Spain studying population genetics of dusky, whiskery and sandbar sharks in Western Australia.

Medical, dental, and veterinary Schools are also involved in elasmobranch studies. The role of parathyroid hormone-related protein in sharks and its possible role in skeletal growth and calcification is being studied at the University of Melbourne, St Vincent Institute of Medical Research. John Clement studies calcium regulation in chondrichthyans at the University of Melbourne School of Dental Science. David Kingston is investigating properties of deep-water dogfish liver oils (glycerols) which stimulate the human immune response at the University of Melbourne Veterinary School. Kenneth Brown studies shark bite patterns and forensic investigations of shark attack at the University of Adelaide School of Dentistry.

The following museums, public aquariums and non-governmental organizations also have an active interest in Chondrichthyans: Australian Museum (Sydney, NSW): John Paxton (Systematics; shark control programs and shark attack); Western Australian Museum (Perth, WA): Barry Hutchins (Systematics), Martin Gomon (Systematics), and Noel Kemp (studies on the dentition of Cretaceous, Tertiary and recent chondrichthyans); South Australian Museum (Adelaide, SA): Ian Beveridge (Systematics and life-history of chondrichthyan parasites); Underwater World (Sunshine Coast, Qld): Rod Garner; Taronga Zoo (Sydney, NSW): John West (Australian Shark Attack File); Western Australia's Underwater World: Bruce Mackay; TRAFFIC (Sydney, NSW): Glenn Sant (Conservation and management); Humane Society International: Bill Foster (Conservation and management); Australian Shark Conservation Foundation: Ian Gordon.

### **Non-invasive studies of elasmobranch fishes using high resolution magnetic resonance imaging**

Geoffrey Waller of The Royal London Hospital, Department of Morbid Anatomy, University of London, United Kingdom presented a demonstration of the application of high resolution magnetic resonance imaging (MRI) to the study of valuable and unique preserved elasmobranchs from the collection of the Natural History Museum of London. Museum specimens are frequently used in systematics and comparative anatomical studies, however a serious limitation is that requests to "dissect" and, therefore, damage the specimen is often denied. These specimens are usually rare and in some instances are the only known specimen of a particular species. The application of non-invasive methodologies such as x-rays, computed axial tomography and ultrasound imaging has proved to be useful. The application of computerized techniques allows the selective acquisition of visual sections of the specimen less than 1 mm in thickness with a resolution of 500-800 microns. Slices may be taken at any plane and the resultant images processed via computerized three dimensional reconstruction methods. This allows the reconstruction to be rotated and examined from several viewpoints. The cost of the imaging is reasonable given the high quality and amount of data obtained. MRI is particularly useful in the study

of muscles and skeletal components. Isolated muscles or parts of them can be visualized separate or combined with their skeletal attachments. MRI was applied for the first time to study a holotype of the only known Ridgeback shark (*Centroscymnus macracanthus*). The 3D relationships of a previously undescribed hypobranchial muscle in the Black shark (*Dalatias licha*) was also presented. The combination of MRI and 3D reconstruction have important implications to the study of functional morphology, phylogenetics and systematics.

#### **Current research activities on elasmobranch biology in Japan**

Kazunari Yano of Seikai National Fisheries Research Institute, Ishigaki Tropical Station, Japan discussed several current research projects from his laboratory and across Japan. In 1990 several shark attacks occurred in Japanese waters which prompted the establishment of a research project devoted to the behavior of sharks and shark attack. Behavioral responses of sharks to several stimuli including sound, smell, visual and electrical impulses were initiated. Species studied by Japanese researchers include rare species such as the megamouth shark, *Megachasma pelagios*, the goblin shark, *Mitsukurina owstoni*, the frilled shark, *Chlamydoselachus anguineus* and the squaloid shark, *Trigonognathus kabeyai*.

#### **Positive and negative outlooks for United States and world shark fisheries**

Frank Schwartz of the Institute of Marine Sciences, University of North Carolina, U.S.A. provided an optimistic view for the future of shark fisheries. Worldwide concerns have steadily increased regarding catches of shark species. A "gloom and doom" atmosphere has developed concerning continued existence of sharks. Such views have led to management plans and implementation for many sharks and world fisheries. Unfortunately most shark management plans are based on a little solid biological data. Extrapolation and mathematical models are frequently used management tools. There is a lack of long term tagging and biological stock assessment data. Most plans also failed to consider short and long-term environmental cycles and species cycles that offset abundance presence and seasonal availability. Model inaccuracy is further compounded by attributing catch data to landing port, not catch site. Limitations on number of fishermen, trip quotas, and seasons have drastically affected commercial and sport fisheries world wide. Several shark fisheries increased in the Atlantic including the Basking shark and Porbeagle and in the Pacific, shortfin mako, spiny dogfish California thresher, California Soupfin and the Angel shark. Following dramatic catch increases in the seventies and eighties and disregarding the biological information, management measures and plans were initiated in the Atlantic and Gulf coasts. Nationally 35 Atlantic, 31 Gulf of Mexico and 27 Pacific oceans species were affected by regional and national plans. Long lining of the same mid-Atlantic shelf stations from 1967 to the present in Shackleford Banks, N.C. have provided data. Abundance of 3 species increased prior to the 1991 management plan. It appears premature to know the effects of the plan as commercial catches have also increased. Post plan CPUE's may be influenced by factors other than the plan. The increased commercial catch quotas have driven many commercial fisherman to their under utilized areas and species. In brief, environmental fluctuations such as water temperature, seasonal timing of sampling and other factors cause interpretation problems as concerns shark fisheries. Dr. Schwartz concludes the shark will persist in spite of man.

### **The precautionary principal and shark management**

John Musick of the Virginia Institute of Marine Science, Department of Fisheries Science, U.S.A. presented a different view from Dr. Schwartz and encouraged the use of the precautionary principal in shark management. He pointed out that most sharks are long-lived, mature at an advanced age and have a small number of young. These vital parameters result in very low rates of intrinsic increase. The history of virtually all unmanaged commercial shark fisheries has been one of rapid stock collapse and recovery. A recent directed fisheries for large coastal shark along the United States Atlantic Coast resulted in a rapid stock decline with little sign of recovery after belated implementation of a fisheries management plan. Although there was no significant density dependent increase in growth rate after stock collapse, or reduction in age at first maturity, survivorship of young-of-the-year appeared to increase substantially. Primary predators of these small juvenile sharks are larger sharks of several species, all of which have been depleted by the fisheries. Monitoring older juveniles may not reflect the true status of the stocks even though recruitment of young-of-the-year may be closely correlated with parent stock size. Shark stocks must be managed using a precautionary approach, because once these stocks are depleted, decades are required for recovery.

### **Total neuron numbers in the electrosensory hindbrain of five elasmobranch species using stereological techniques**

Sara Metcalf of the Marine Studies Department, Bay of Plenty Polytechnic, Tauranga, New Zealand examined the brain size and total neuron numbers in five elasmobranch species and analyzed them to investigate differences and the size and neuron structure of the dorso-octavolateralis electrosensory nucleus (DON). The variation in brain size and neuron numbers were matched with other correlates such as taxonomic relationships and migratory behavior to investigate whether these properties could help explain differences in a sensory system believed to be multi-functional with respect to prey and navigational ability. Using reliable stereological methods total neuron numbers in the DON of elasmobranchs has been obtained for the first time. A significant proportion of the brain size variation was attributed to variation in body size but total neuron numbers of the DON did not correlate well with either body size or peripheral ampullae receptor numbers.

### **Current research on chondrichthyes in France**

Bernard Séret of Antenne ORSTOM, Muséum national d'histoire naturelle, France traced the history of ichthyological studies by French naturalists from the coasts of France and their overseas territories and former colonies. France has been active in investigating various aspects of the biology of sharks, skates and rays from the time of Risso in 1810 up to the development of SCUBA by Jacques Cousteau. Several researchers are investigating fishes in general in France but few are currently focusing in elasmobranchs exclusively. Systematics is being investigated at the Muséum national d'histoire naturelle in Paris and ORSTOM in New Caledonia is also active.

### **The role of non-governmental organizations in networking for research, management and conservation efforts**

Sarah Fowler, IUCN, Nature Conservation Bureau, United Kingdom and Merry Camhi, IUCN Shark Specialist Group, National Audubon Society, U.S.A. concluded the workshop by discussing the role of non-governmental organizations in networking for

research, management and conservation efforts. They emphasized the increasing need for sustained dialog between governments, research institutions, conservation groups, game fishing associations and management agencies. Elasmobranchs, especially sharks, have drawn attention within CITES (the Convention on International Trade in Endangered Species). CITES has no management authority but is restricted to conservation issues as they relate to international trade. Management is the obligation of domestic fisheries and wildlife agencies or regional and international management authorities. The IUCN (the International Union for the Conservation of Nature and Natural Resources, also known as the World Conservation Union) is the umbrella organization of the world's conservation agencies. Members come from 65 sovereign states, 99 governmental agencies and 588 non-governmental organizations. The Shark Specialist Group was established by the IUCN in 1991 and has become a leader in providing expert data and recommendations related to shark issues. Networking between involved and interested groups is a major goal of international cooperation. Organizations such as the American Elasmobranch Association and sister groups in other countries serve as a mechanism for dialog and dissemination of information on elasmobranchs, as well as a forum for discussion of pertinent issues. Many of these groups maintain web pages and sponsor Internet bulletin boards devoted to elasmobranchs. Such organizations as IUCN and the National Audubon Society serve as valuable sources that can provide network links to organizations, institutions, agencies and individual researchers involved in various aspects of elasmobranch biology.

#### **Status of elasmobranch research in Brazil**

Alberto Amorim of the Instituto de Pesca, Divisao Pesca Maritima, Santos, Brazil was unable to attend the workshop due to a last minute difficulty with travel documents but I would like to briefly present what would have been the substance of his presentation. Dr. Victor Sadowsky (1909-1990) was the foremost elasmobranch biologist to consider Brazilian species. He published 16 scientific papers and 25 abstracts while working at the research laboratory of the University of Sao Paulo located at Cananeia. Most of his work dealt with shark and ray identification, tag and release, reproduction and shark attack. In January 1985, the Brazilian Shark and Ray Group was founded and the first workshop was held at the Instituto de Pesca in Santos with 50 registrants and 10 presented papers. The second workshop was held in Sao Luis, Maranhao in 1986 with 14 presented papers. Subsequent workshops showed a rapid increase in the number of contributed papers: Fortaleza with 18 papers, Recife with 31 and Santos with 35, Recife with 49 and Rio Grande with 62. In July 1997, the Brazilian Shark and Ray Group was transformed into the Brazilian Elasmobranch Society (SBEEL). The first meeting in the same year had 74 papers and about 100 members. Topics included reproduction, biogeography, age and growth, conservation and management, morphology, commercial fisheries and food, genetics, microbiology, parasitology, histology, shark attacks, systematics and pathology. Established elasmobranch research programs exist at several locations throughout Brazil. The program in Sao Paulo concentrates on fisheries biology of pelagic sharks and shark attack. Work in Rio Grande at the Fundacao Universidade de Rio Grande stresses the biology of commercial sharks and rays. The program at the Universidade do Estado do Rio de Janeiro is concerned with fisheries biology of coastal and pelagic sharks. The Universidade Federal da Paraiba also has several projects. Principal species studied at the various sites include *Prionace glauca*, *Carcharhinus signatus*, *Rhizoprionodon*, *Sphyrna*, *Mustelus*, *Rhinobatos* and *Squatina*.

## CONCLUSIONS

In summary, elasmobranch fishes deserve continued attention by scientists for several reasons. They play a key role in the ecology of the oceans, are a valuable fishery resource and provide a multitude of opportunities for laboratory based investigations. The level of interest in elasmobranchs worldwide is at an all time high. Everyone from school children to scientific professionals are aware of the pivotal position of elasmobranchs, in particular sharks, in the dynamics of the ocean. Concerns ranging from ocean pollution, global warming to over fishing have direct effects on elasmobranch populations and public interest in elasmobranchs may be used to increase awareness of these major environmental concerns. Scientific inquiry in these areas is vital to insure continued healthy stocks. Elasmobranchs are elegant models in which a variety of phenomena of interest to cell biologists, physiologists and biochemists can be explored. Interest in the biology of elasmobranchs will continue to grow.

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## MAGNETIC RESONANCE IMAGING STUDY OF THE HOLOTYPE OF *CENTROSCYMNUS MACRACANTHUS* REGAN, 1906 (SQUALIFORMES: SOMNIOSIDAE)

by

Geoffrey N.H. WALLER (1)

**ABSTRACT.** - The holotype of *Centroscymnus macracanthus* was used for experimental analysis with magnetic resonance imaging and three dimensional computerised reconstruction techniques. Internal structures of the head and body cavity are described for the first time and considered in relation to the taxonomic position of this shark. Development of these techniques for computer-based archival purposes will greatly increase the resource value of collections of formalin-preserved specimens, mainly the types, such as those in museums.

**RÉSUMÉ.** - Étude de l'holotype de *Centroscymnus macracanthus* Regan, 1906 (Squaliformes, Somniosidae) en imagerie par résonnance magnétique.

L'holotype de *Centroscymnus macracanthus* a été utilisé pour une analyse expérimentale d'imagerie par résonnance magnétique et pour tester trois techniques de reconstruction tridimensionnelle par ordinateur. Les structures internes de la tête et de la cavité abdominale sont décrites pour la première fois, et en relation avec la position taxinomique de ce requin. Le développement de ces techniques d'archivage documentaire informatisé augmentera la valeur des collections des spécimens, notamment des types, fixés au formol, comme ceux qui sont conservés dans les musées.

**Key-words.** - Elasmobranchii, Squaliformes, Somniosidae, *Centroscymnus macracanthus*, Magnetic resonance imaging, Three dimensional reconstruction, Computer-based archiving.

The use of high resolution magnetic resonance imaging (MRI) to study the internal structure of fixed elasmobranch fishes is a revolutionary new advance (Waller *et al.*, 1994) in the field of biomedical imaging. The combination of high resolution MRI with computerised three dimensional (3D) reconstruction techniques enables the "dissection" of a specimen in any plane on the computer screen. It allows 3D representations of selected internal anatomical features to be displayed in multiple orientations and enhanced with computer-generated colour renderings (Waller and Cookson, 1996). The feasibility of using these techniques to elucidate the soft-tissue structure of elasmobranch holotypes was investigated here; the invasive nature of standard anatomical methods (e.g., staining, dissection) precludes their use on holotypes. The low radiographic contrast of elasmobranch cartilage makes finer skeletal elements difficult to resolve. In this study, the holotype and only known specimen of the largespine velvet dogfish (*Centroscymnus macracanthus*) was chosen for experimental analysis.

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## MATERIALS AND METHODS

The holotype of *Centroscymnus macracanthus* is a female 67.5 cm in TL, preserved in the collections of the Natural History Museum, London (BMNH-1884.2.6.7). It was caught in the Magellan Straits some time before 1906 and has been stored in 70% ethanol. All data were acquired with a GE 1.5T scanner (IGE Medical Systems, Milwaukee, U.S.A.) with a 60 cm bore and a 23 mT/m gradient set. Two local coils (3" circular coils) tuned to an operating frequency of 63 MHz were used to detect the RF signal.

Contiguous slices were obtained serially across the full width of the specimen in the sagittal plane (i.e., parallel to the long axis of the specimen) and each scan consisted of 119 slices. Between 8 and 40 averages were used with a data collection times of 8.5-9.0 h for the spin echo sequences. For gradient echo sequences, seven averages were used with a total scan time of 32 min; 128 slices were collected in the sagittal plane. Slices in three orthogonal planes were obtained during post-processing of the raw data sets. Resolution was 750  $\mu\text{m}$  x 750  $\mu\text{m}$  in the x/y dimension and slices were 0.75 mm (gradient echo) and 1.5 mm (spin echo) in thickness.

Susceptibility effects were found to be reduced by using data collection methods with smaller voxel sizes. Optimal slice thicknesses of 750  $\mu\text{m}$  were achieved during the experiments reported here. Tissue contrast resolution was maximised using a T1-weighted gradient echo imaging sequence with an inversion preparation pulse. Three dimensional reconstructions were made with the ANALYZE software (Robb and Hanson, 1990).

## RESULTS

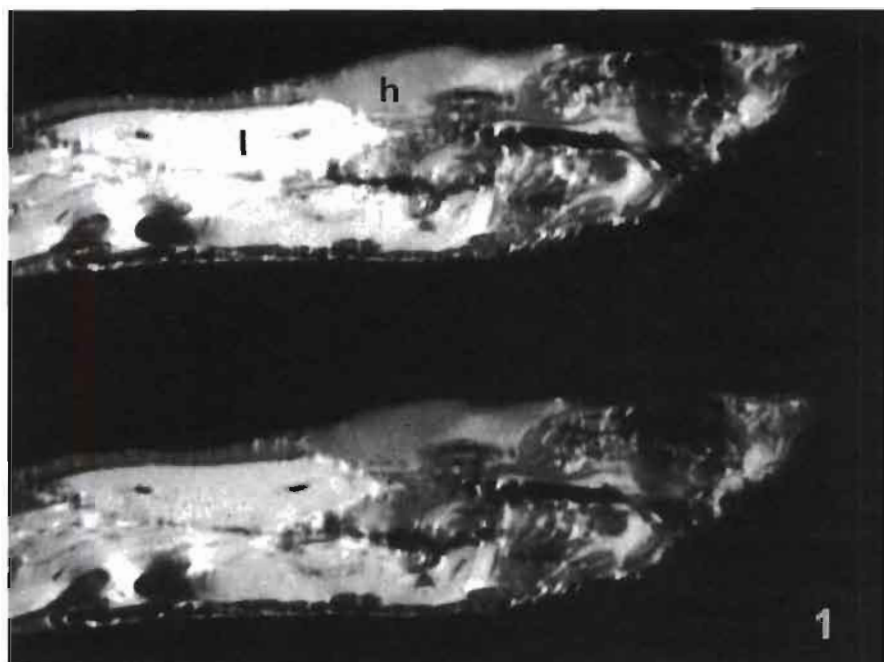
The images obtained successfully demonstrate the external and internal anatomical features of *C. macracanthus* in two dimensional (2D) images and in 3D reconstructions. Tissue differentiation was sufficient to distinguish between hypaxial muscle, skeletal cartilage and gastro-intestinal tract (Fig. 1). In both T1- and T2- weighted images, liver tissue gives a 'bright' signal and this may be due to a high fat content. The spinal column shows clearly differentiated centra in the MR images; evidence for a 'notochordal' condition in which the spinal column shows reduced segmentation was not observed here. Central nervous system tissue is generally not well preserved except superficially in the olfactory capsules where the olfactory epithelium shows a characteristic topography (Fig. 2); proximal olfactory peduncles are also evident. The chondrocranial features indicate a well developed optic capsule supporting the large eyes. A short rostrum is present between the well chondrified nasal capsules.

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Fig. 1. - Adjacent spin echo MR images (TR/TE: 8000/48) through the anterior body of the holotype of *Centroscymnus macracanthus* (BMNH-1884.2.6.7). Sagittal sections 1.5 mm thick lateral to midline. Note prominent liver tissue (l) filling body cavity in this section plane, and contrast to hypaxial muscle tissue (h).

Fig. 2. - Horizontal (left) and sagittal (right) gradient echo (with inversion preparation pulse) MR images (TR/T1/TE 14/200/4.2) of the head of the holotype of *C. macracanthus* (BMNH-1884.2.6.7). Slice thickness 750  $\mu\text{m}$ . Well developed cartilaginous septum between nasal capsules (s); e, eye lens; l, liver; o, olfactory rosette; v, vertebral column. Spiracle position arrowed. Note the enhanced contrast resolution in this 3D volume data set when compared to figure 1.





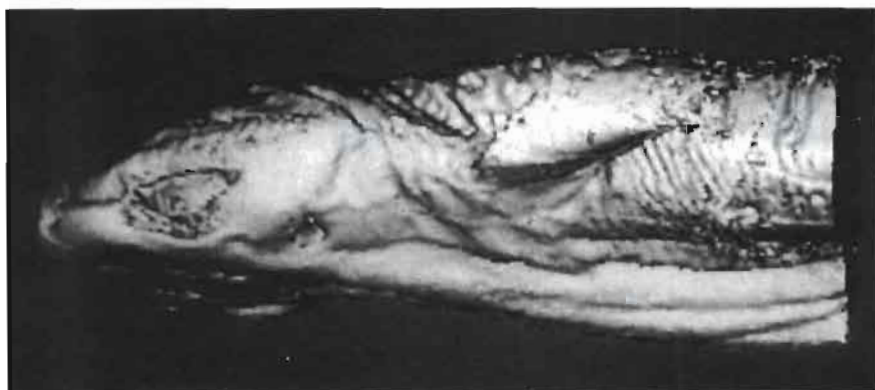


Fig. 3. - Three dimensional reconstruction showing external morphology of the holotype of *C. macracanthus* (BMNH 1884.2.6.7). Spin echo images (TR/TE 300/13), slice thickness 1.5 mm. Note ridge at the dorsal midline. Pectoral fin lies against flank, spiracle evident behind eye.

The external morphology of the holotype is shown in the 3D reconstruction (Fig. 3) in lateral view. The image shown has been optimally thresholded to show the integument but dermal denticles were not resolved. A dorsal ridge extends forwards along the back to a point level with the base of the branchial region. Posteriorly it merges with the front of the first dorsal fin.

## DISCUSSION

Preserved ichthyological type specimens in museum collections are a valuable scientific resource. However, the scientific potential of type material is currently limited by the difficulty of investigating internal structure and their unsuitability for molecular studies (due to formalin fixation). This is particularly the case where taxa are rare or difficult to obtain and available material can not be used for exploratory dissection, staining or other invasive studies. For those taxa represented by single specimens, exclusive use of non-invasive techniques is required. Here, for the first time, a computer-based technique is described that allows both external and internal anatomical features of unique specimens to be accessed without reference to the original specimen. Anatomical features can be viewed as 2D images and as 3D reconstructions. Once the MRI data set has been prepared, the technique has the advantage of enabling multiple observations on the same specimen to be made whilst avoiding the problems of specimen damage inherent in standard techniques. The results from both 2D and 3D analysis can be used to distinguish new anatomical characters for phylogenetic analysis.

Museum type specimens stored in ethanol are subject to varying degrees of tissue abrasion, and this can be apparent particularly in more frequently accessed specimens. Delicate superficial structures such as lateral line scales (for example in Lizard Fishes, Synodontidae) or photophores (for example in Lantern Fishes, Myctophidae) are easily disturbed or lost during handling or as a result of the effects of repeated drying and wetting

when specimens are removed from storage for examination. Both photophores and lateral line scales are of diagnostic importance in these and other teleost families. It is apparent therefore that current wet-storage methods, which are in world-wide use in museums and private collections, will not be sufficient to ameliorate the effects of handling in the longer term. Computer-based archives used for storing renderable and segmentable 3D image datasets of type material represent one possible solution here. The 3D data sets used in this study were small, typically 20 Mb, thus making storage and retrieval of 3D images feasible on standard PC networks.

The rostrum of *C. macracanthus* is short and comparable to that of the related short-snouted co-generic species *Centroscymnus coelolepis* (MRI, pers. obs). However external morphological features of *C. macracanthus*, notably micro-squamation of the denticle covering and large fin spines, are not characteristic of the genus *Centroscymnus* as currently defined (Compagno, 1984). There are no data currently available on the chondrocranial structure of the other short-snouted *Centroscymnus* species (*C. owstoni*, *C. cryptacanthus*, and *C. plunketi*) referred to this genus (Last and Stevens, 1994) which could be used as a basis for comparison with *C. macracanthus*. It is noteworthy that these three species have short fin spines and heavy skin denticulation. Resolution of the correct generic position of *C. macracanthus* must await further MRI studies on the chondrocranium morphology of this holotype and other related taxa.

**Acknowledgements.** - David MacManus provided invaluable technical assistance and supervised scanning of the specimen. Dr P. Tofts is thanked for providing access to MRI facilities at the Institute of Neurology, University College, London. Oliver Crimmen is thanked for providing curatorial assistance at the Natural History Museum, London.

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## Workshop 8

### RELATIONS BETWEEN MAN AND FISH IN SOUTH PACIFIC CULTURES

Chairperson: **Marie-Claire BATAILLE-BENGUIGUI**

BATAILLE-BENGUIGUI M.-C. - Men/fish relationships in traditional societies, their effects on human behaviour. 849-860

*Les relations homme/poisson dans les sociétés traditionnelles; leurs effets sur le comportement humain.*

PALOMARES M.L.D. - On the biological information content of common names: A quantitative case study of Philippine fishes. 861-866

*Sur l'information biologique contenue dans les noms vernaculaires: une étude quantitative sur les poissons des Philippines.*



## **MEN/FISH RELATIONSHIPS IN TRADITIONAL SOCIETIES, THEIR EFFECTS ON HUMAN BEHAVIOUR**

by

Marie-Claire BATAILLE-BENGUIGUI (1)

**ABSTRACT.** - Within the framework of the study of the relationships between man and animals, the fish have been forgotten; one of the reason being that they move in an environment that is not ours and is considered as "wild", "not domesticated", or more exactly which escapes from man's control. Now, in the whole world and at all times, from antic Greece to the Pacific, from Africa to Charlotte island in the north-west coast of America, we have examples of privileged relationships between men and specific ichthyological species, provided that we are of course set in a non industrial context. Many examples are provided by myths, oral and written tradition, daily life, materialistic representations and those of the social and religious imagination, and this in freshwater as well as in marine water. For the societies of the Indo-Pacific area, land and sea form a continuity, a reticular woven area in which men, islands, fish and gods intercross, and have something to do with each other. The shore does not constitute a rupture but rather a link within which people of the land and people of the sea have exchanges, complete each other and realize themselves. In these societies, fish is often perceived as a social partner, which makes adopt specific behaviours concerning the appropriation of the sea resources, including rituals totally associated with techniques. This privileged relationship with ichthyological species associated with a strict sea tenure, which means controlling the access to fishing areas - a part often filled by the local traditional hierarchy - creates a balance between man and its surroundings, and leads to the respect of ichthyological species, environment and nature on the whole. Putting together crossing eyes, different perceptions and feelings in the perspective of departing subject matters deserves a meeting between hard sciences and human sciences.

**RÉSUMÉ.** - Les relations homme/poisson dans les sociétés traditionnelles; leurs effets sur le comportement humain.

Dans le cadre de l'étude des relations homme/animal, les poissons sont oubliés, entre autres raisons parce qu'ils évoluent dans un milieu qui n'est pas le nôtre, considéré comme "sauvage - non domestiqué" ou plus exactement qui échappe au contrôle de l'homme. Or dans le monde entier et de tous temps, de la Grèce antique au Pacifique en passant par l'Égypte, de l'Afrique à l'île Charlotte sur la côte nord-ouest américaine, on a des exemples de relations privilégiées entre les hommes et des espèces ichthyologiques spécifiques à condition de se placer dans un contexte non industriel bien sûr. De nombreux exemples sont fournis par les mythes, la tradition orale comme écrite, le vécu quotidien, les représentations matérielles et celles de l'imaginaire social et religieux, ceci dans les eaux douces comme salées. Pour les sociétés de la région indo-pacifique, terre et mer forment une continuité, un tissu réticulaire où hommes, îles et poissons se croisent et s'interpellent; où le rivage ne constitue pas une rupture mais un lien, où "gens de mer" et "gens de terre" échangent, se complètent et se réalisent. Dans ces sociétés, le poisson est souvent perçu comme un partenaire social, ce qui entraîne de la part des hommes des comportements particuliers dans l'appropriation des ressources de la mer avec des rituels associés aux techniques à part entière. Cette relation privilégiée avec des espèces ichthyologiques, associée à une rigoureuse tenure maritime, c'est-à-dire un accès aux lieux de pêche contrôlé par une hiérarchie traditionnelle locale, crée un équilibre entre l'homme et le milieu, un respect des espèces

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ichthyologiques, de l'environnement et de la nature en général. Une mise en commun de regards croisés, de perceptions et d'expériences, dans une perspective de décloisonnement des disciplines mérite une rencontre entre partenaires issus de la biologie et des sciences humaines.

Key-words. - Fishing practice, Anthropology, Oceania, Environment, Mental representations.

Why would "men" suddenly appear in a conference about the "fish" from the Indo-Pacific area and some social sciences in an assembly of biologists and ichthyologists? Maybe you asked yourself this question when you received the program of the 5th Conference which brought us together at Nouméa. The story began during informal corridor conversations in the Laboratory of Ichthyology at the National Museum of Natural History of Paris in 1995. Indeed, as an ethnologist, I had studied the rituals which accompanied some fishing techniques and some ichthyological species from Polynesia and I also spent several years in this laboratory by accident. I discovered the work of the systematists and a new reality of aquatic animals which might, in the long term, allow me to comprehend the mental illustration the Polynesians had of them. For my part, I tried hard to point out the fact that if one made the effort to depart mentally from the strict lab work, those smelly fish jars and *calculation of the number of spines in the back of the mexican scomber* for instance (Steinbeck, 1951 - the way this book alternates emotional and scientific registers was an influence on how I approached the subject and enabled me to dare to exchange ideas with specialists in the biological sciences) there existed another approach to "fishness", that is sensitive and psychological and that both of them combined would get us closer to the reality of the world. I also thought that the representations of the imaginary which are linked with fish in different societies could have an influence on the systematics applied to fishing and that exact and human sciences could be complementary approaches to the fish subject and the realities of aquatic space in fresh, brackish or seawater.

These suppositions turned out to be accurate when I was able to exchange information with B. Séret about sharks, a sacred animal if ever there was one, in Oceania. The physiology of sharks that I discovered in the laboratory enabled me to work out a better interpretation of the myths that I had collected and the way Polynesians behaved towards these Leviathans of the South Seas. I also thought, perhaps naively, that the information gathered about the relations people maintained with these fish and the world of the sea in these tropical island societies - but also in other societies and environments - might improve modern fishing methods, as well as enhance respect for biodiversity of species and for the environment. The new light cast on this subject could also make it possible to refine development projects involving fishing which would take into account both human social life and that of ichthyological species, as well as limit the damage entailed by poorly managed "prey-predator" relations. Bauchot (1967) studied various forms of the social life of fish which had previously been relatively neglected and which bring out the fact that *the world of vertebrates has social structures that are less specialized than those of insects, but more closely related to those of human societies, or at least which reveal behavior complexes that are easier for us to understand....the quintessence of the social life (of fish) is a permanent school* (p. 17 and 143). Deeper consideration of these relational aspects should lead to respect, conservation and better management of biodiversity (Lévêque, 1997). This is how this workshop came to be proposed under the title of *Relations between man and fish in south Pacific cultures* in order to explore the possible inter-



actions between social sciences and fishery sciences. According to the people in charge of the organization, the response was favorable, although few people signed up for the workshop.

After tracing the development of this workshop, I shall attempt to explain briefly the history, importance, extent and effects of relations between men and fish in different cultures of the world that have often gone unnoticed or been neglected. Then, I shall take up some specific examples of men/fish relations in Polynesia.

### **RELATIONS BETWEEN MEN AND FISH. SOME INFORMATION IN THREE POINTS**

1. First of all, the multiplicity of representations of fish or live marine elements that we can observe on (or in) the shape of different objects of material cultures of the world cannot be without meaning. It cannot be only a coincidence, a decoration, the representation of part of the environment used in an aesthetic perspective. We have to find the reasons and some explanations or to formulate hypotheses.

I can only present a few examples out of many of them of the way fish are represented in material artefacts from various places in the world in order to illustrate my analysis (Fig. 1).

On the one hand, these representations of fish either found on objects or determining their form belong to a code of correspondance that is more or less complex in each society mentioned as regards these material productions. They illustrate the impact of fish on the imaginary world of people and are often the reflection or the mythical background or expression of part of their social and religious organization. On the other hand, the vast realm of oral literature, which I can only mention in passing here, treats the fish as a mythical hero, even quite often in the feminine (Krauskopff, 1987). Myth is often the basis of these material representations. It is well known that fish are the symbol of water, of life, fecundity and wisdom, even of good luck, all over the world, in Christianity as well as in many other religions (Chevalier and Gheerbant, 1969). In prehistoric art, fish, although numerically secondary in rock art and artefacts in comparison with pictures of quadrupeds, are definitely present and evocative of a symbolism and mythology still poorly understood (Cleyet-Merle, 1990).

2. If man/animal relationships have been extensively studied in recent decades in the field of ethnoscience and more precisely in ethno-zoology, it has been relations between men and terrestrial animals. The representations of horses, bears, some kinds of pets such as dogs and cats, insects or birds and so on have gradually become understandable and the boundary between man and animal has become blurred. But not much has been done on the signification of the relations between man and aquatic creatures in this field, either in salt, brackish or fresh waters. If terrestrial animal "bestiaries" abound, there is nothing much on aquatic animals. Can we explain that by the fact that man is not familiar with the aquatic environment (even if his ancestors long ago emerged from it) and also not familiar with what appears to be a wild and unknown place outside the controlled forces of nature? If I may indulge in some unelevated irony, I would say that anthropologists probably do not know how to swim very well, so we prefer to study what is going on on land more than on what is going on in the water. I had better stop here to formulate some questions and try to cast more light on the subject with examples taken from traditional societies in particular circumstances.



Fig. 1. - Material representations of "sacred" fish.

**A:** Shaman's guardian figure with his tutelar spirit in a shape of a fish. Tlingit tribe. Northwest coast of Canada. Painted wood, 40 cm. Coll. Musée de l'Homme N°85.78.34, cliché M.H.; **B:** Piece of cloth with painted fishes coming from a precolombian tumb. Pérou, central coast, Chancay culture, XIe-XVe century. Coll. Musée de l'Homme N°11.21.449; **C:** Stone carp used as a gong to call people at prayer. China, "White Cloud" temple near Pékin. Cl. Pierre Colombel.



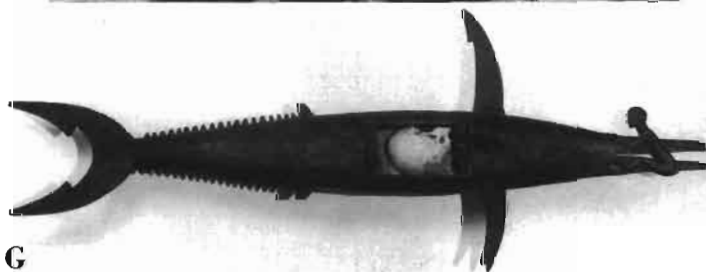
D



E



F



G

Fig. 1. - Material representations of "sacred" fish (continued).

**D:** Shark god. Santa Cruz, Salomon islands. Representation of an ancestor kept in the fishermen house where are performed rituals before going fishing in open sea. Wood with smocky sheen, 17 cm. Coll. Musée de l'Homme N°69.51.25, cliché M.H.; **E:** Shark, emblem of the King of Dahomey. Bénin, western Africa. This animal representation magnify the King. Painted wood, 160 cm. Coll. Musée de l'Homme N°93.45.3, cliché M.H.; **F:** A man worship a *Mormyrus niloticus*, sacred fish in Egypt. Saïte period, VIe-Ve century b. J.C. Bronze, 30 cm. Private collection; **G:** Skull ossuary. Santa Ana, Salomon islands. Skulls were put in a box shaped as a shark fish for the second funerals. Bonito and shark are sacred fishes in nearly all Oceania, chiefs and ancestors are often incarnated into them. Wood, 208 cm. Coll. Musée de l'Homme N°61.103.56, cliché M.H.

3. As an illustration of my purpose, I propose to focus on this type of relations in a society where I studied this subject not far from here, approximately two thousand kilometres east of New Caledonia, in the Kingdom of Tonga. The archipelago of the Tonga Isles, nicknamed the Friendly Isles by Captain Cook in 1773 because of the friendliness of their inhabitants, is located in the southern hemisphere near the islands of Fiji and Samoa. It is made up of 170 islands, only 36 of which are inhabited today by 95,000 people. The total dry-land surface area is 700 square km, spread out over 800 km from north to south within an exclusive economic zone (or "the 200-mile zone") of 360,000 square km. The inhabitants of these islands came there by sea, they always relied on it for subsistence resources, means of communication, sources of imagination, etc. Tonga is the only place in the Pacific which was never colonised and was only under a British protectorate from 1900 to 1960. It was the first kingdom in Polynesia and remains the last one with King Taufa'ahau Tupou IV. If the first settlement goes back to 1500 B.C., according to archeologists, oral tradition tells us that the first king is known by 950 A.D. He is the son of a god and a human woman, and the whole land belongs to him because of this divine origin. This means that no foreigner had been able to acquire some land in the country until the present time but could only lease land. This historical and political context has preserved tradition more than anywhere else in Polynesia. This is why it has been a good laboratory in which to study the subject, with small islands that are still fully Polynesian.

Social organization is very hierarchical with three successive royal lines, chiefs, masters of ceremony or talking chiefs (*matapule*) and commoners. The concept of "superior/inferior" between individuals regulates political as well as domestic areas.

We find part of this order reflected in different categories of fish: some are kings and sacred or social partners, the others are common fish, ordinary prey, good only for consumption. The former are treated like men or gods and the others like things. The relation between men and fish exist on several levels, where the *personnification/reification* polarity is brought out in different ways" (an expression borrowed from Sigaut, 1995). Some rituals are attached to the fishing techniques used to catch the former categories, while the others are caught in ordinary ways (Bataille-Benguigui, 1992, 1994). Those differences of status for fish are met with in many other areas of the Pacific.

First of all, in Tonga as in other islands of Oceania, the island itself is a fish, because, according to the myth of origin, some lands were fished up by the god Maui from the bottom of the sea with a mythical hook. Belief in Maui is spread from Micronesia to New Zealand, from Tikopia to Easter Island. Let me add that even man is a fish according to lore from another place in the Pacific. In Eddystone, in the New Georgia group (Solomon islands), as part of chiefs' funerals, the head is set on an altar and surrounded with shell rings, which are used as traditional currency. They shape the mesh of a net in which is caught the head of the dead chief who is assimilated to a fish so that he becomes an ancestor. The souls of dead people are treated as fish and captured with a net to send them to the world of the dead (Barraud, 1972).

Coming back to Tonga, among the numerous gods of the pre-Christian pantheon, many of them are incarnated in fish, they become the *vaka* (boats) for the gods to visit human beings and the priests visited by those gods and playing the role of mediators with human beings are called the *taula* (anchors) of the gods. They anchor the god's breath or a divine presence in the human world. All the cosmological concepts are permeated with an oceanic dimension, with sea and aquatic creatures.

Sacred fish, subjects of taboo and rituals in certain places of the archipelago, are also social partners. They are, in order of importance:

- any kind of shark (*anga*), specially in the waters east of the main island Tongatapu;
- the hammerhead shark (*matai*) in Tongatapu lagoon waters;
- the bonito (*atu*, *Katsuwonus pelamis*), in front of Ha'ano beach, a village in the Ha'apai group of islands;
- the goatfish (*vete*, *Mulloidichthys vanicolensis*) at the village beach of Niutoua on Tongatapu;
- the milkfish (*ava*, *Chanos chanos*) in the brackish waters of the lake of the island of Nomuka;
- the *pelupelu*, an unidentified sort of herring in the waters of Atata island.

According to their myth of origin, most of them are love gifts from *Pulotu*, the Tongan paradise, which is a mythical island unknown by any human being. They are all the property of a local chief and are fished ritually in their village. Anywhere else, they are ordinary fish, treated without special attention or consideration, there is no need to observe any taboo and they are considered ordinary prey fished for consumption.

Sharks are noosed with a slipknot, metaphorically seen as a necklace of flowers, called by the fishermen from the boat with a rattle made of coconut husks and treated as a fiancé called Hina like the Polynesian goddess with a metaphorically high-voiced language. They are given some bait qualified as royal food. This shark is a woman of royal or divine blood. The bonito in Ha'ano is also a feminine creature called Hina.

Only men go fishing in the open sea, while women stay on land and collect fish and other creatures in the coastal area. Before going fishing, especially for shark, considered as a woman, a man should not have sexual intercourse with any women. They think that the fish will smell the feminine scent and avoid the fishermen. In Micronesia, men even rub their bodies with a special kind of grass to take off any feminine smell and, according to Johannes (1978), "*the sea is a jealous woman*". For this reason, in Tonga, men going to fish shark stay for a few days in a *fale siu*, a taboo fishing house on the beach, away from women, in order to purify themselves before going out to sea. Women and people of the village have to avoid this taboo house the whole time of the fishing. When the fishermen are back with their prey, they break the taboo by sharing some food specially prepared for them in the *fale siu* and drink kava, a ceremonial beverage made of the roots of *Piper methysticum*; then they prepare different shares of fish which will be offered according to the hierarchical order, first to the chief of the village or the island, then to the priest, then to the sister of the head fisherman as in Tongan society, the sister is above the brother, even above the law, another concept which rules social relations. The rest is shared between the fishermen for their families. In any case, the meat must not be sold as shark is a divine fish, a social partner, a woman, that is part of what is forbidden. After that, everything goes back to normal. This taboo on selling is extended to the other "sacred" fish mentioned. If the taboo were broken, the fish would not come back the following years.

Shark accidents happen only to men who are guilty of some offense according to tradition or Christian religion and the shark is perceived as a judge, who reestablishes justice and order in human society. Honest men never have problems and need not be afraid, but guilty men can be judged by the shark.

The hammerhead shark in Nukuleka, the bonito in Ha'apai and the goatfish in Niutoua, are fished near the beaches, so this does not present any risk and rituals are associ-



ated with fishing techniques. When people see them coming they shout so that the village chief will hide himself in his house for the duration of the fishing operations. He can come out only when his share is brought to him. Then the rest is shared exclusively inside the village and must not be sold. This does not agree with the theory of Malinowski on propitiatory magic in fishing. He says that the farther you go and the more risks involved, the more you become anxious and practice magic to ensure yourself of protection and success. In the case of Tongan fishermen, they practice rituals even when there is no risk at all, as an exchange with nature, according to my hypothesis.

In general, the whole population of a coastal Tongan village is qualified as *siu* people, people who fish. You have the *siu tahi*, fishermen of the sea, the men who go fishing and the *siu uta*, people of the land, which means the men who are too old to go out in the open sea, women who are not allowed to go in this men's area and children who stay on the land while the men go out. It means that fishing, contrary to appearances of being only a men's field of activity, is a mentally collective business. It is done by men but shared mentally by the whole community. When men go out fishing, the rest of the people in the family or in the village, must not make noise and have fun. If somebody dies, if adultery is committed, the men on the sea will fail and have to come back. In fact any climate of social disequilibrium on land will make fishing a failure. We found the same attitude, if a member of the crew goes out fishing after doing something wrong that nobody on board knows about. He would have to make a public confession, if he does not want to ruin the fishing prospects.

There is a second aspect of fishing that I want to approach now - access to fishing areas. In such a hierarchical society, until the end of the last century, access to marine resources and fishing areas was controlled by the king and the chiefs and distributed according to gender and social status (Figs 2, 3).

Since the first and only constitution of 1875 granted by King Tupou I, access to the sea has been free for all men. Women still do not go fishing in the open sea even if recently they have started thinking of doing it and applied for development programs which did not succeed. But today in a crew, if one of the members insists to go on going fishing when the weather conditions are not good, the others would say that he is *tangata ma'anumanu*, an "avaricious" man who wants to have more than the others and would disapprove of him saying that "he want to abuse nature". They will not follow him and will exclude him from the group. It means that access is free but that you must behave in such a way as not to disturb the balance in interrelations between men and between men and nature.

I could add many other details concerning the relations between men, the sea and fish, but it is time to sum up this Tongan testimony. Briefly, in circumstances still existing outside of any idea of profit and commercial utility, the principal effects of those relations are the following:

- fish play a role in the social order and underwrite solidarity, equilibrium and consensus in the family and the village community; fish symbolize harmony and therefore life and act symbolically in the therapy of conflict in human areas (Bataille-Benguigui, 1993):
- some fish acts as mediators between men and gods and as officers of the law;
- the controlled access to resources (still in effect in certain isolated islands of Micronesia - Teikaki, 1988) and the taboo on selling fish prevent overfishing and disrespect towards the natural resources and environment;

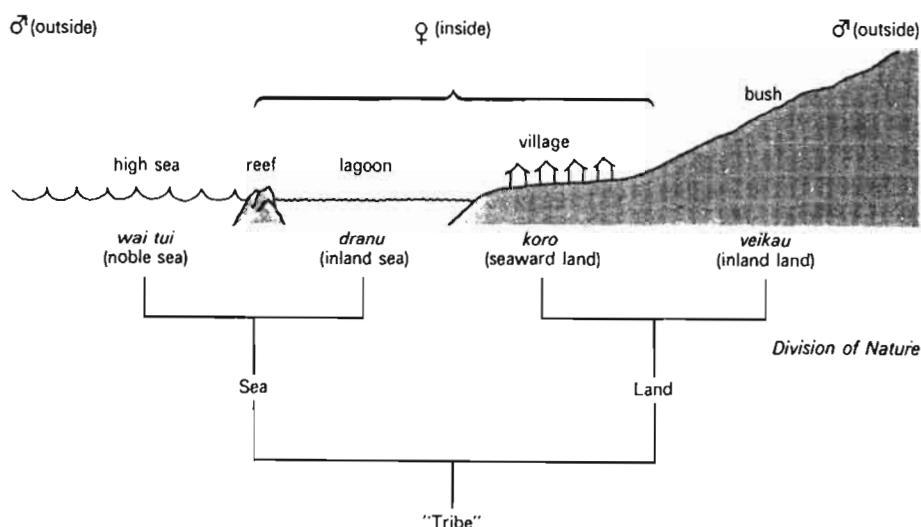


Fig. 2. - Division of labor and division of nature in Fiji  
(Sahlins representation for the Moalans, 1976, p. 40.)

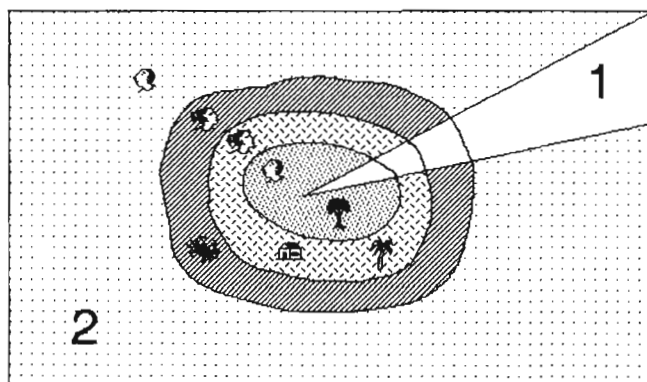


Fig. 3. - Sexual and social division of work and nature in Tonga.

(My representation for Tonga: same sexual division of nature with women in seaward land and inland sea and men in "gardens" and open sea (2) and chiefs having a section of land and sea (1).)

The facts I have described may explain why representations of fish figure on war clubs, on drawings made on bark cloth and that they have inspired danse and poetry, common types of artistic expression in this society based on oral tradition.

The demonstration I have tried to make with Tongan informations can be similar in other societies of the Indo-Pacific area and many other insular societies in the world based on an aquatic type of economy. You also find some taboo in cold water countries and anthropomorphised relations between men and fish in many places of the world. They should be studied before industrial fishing completely replaces traditional and small-scale fisheries.

Before concluding, I want to say a few words about some experiments that were carried out at the University of Pennsylvania and Brooklyn college in New York in 1983 by a team of American medical doctors and biologists, among them Pr Aron Katcher. The group demonstrated that when people waiting for dental surgery, for example, and felling anxious, or children submitted to psychotherapeutic interviews were placed in front of an aquarium, their blood pressure and heart rate went down, thus lessening the anxiety of both adults and children. Their interpretation of this anxiolytic effect is that the sight and the sound of living aquatic animals in running water has a physiological effect on men. The results had already been put into application with aquariums in hospitals, prisons, waiting rooms and other closed-off areas (policy set by M. Hignette, Director of the Aquarium of the MAAO, Musée national des Arts Africains et Océaniens, Paris), intended to have soothing effects. Other positive effects were observed, too: watching fish eat can help children to recover their taste for food; observation of reproduction of parental behaviour and the notion of family in the *Cichlidae* family can reestablish a certain equilibrium in adolescents with problems due to their family or social environment (P. Bouvier, Administrator of the AMAO, Association des Amis de l'Aquarium du MAAO, pers. comm.). What was an aesthetic decoration became a useful and meaningful therapeutic tool.

## CONCLUSIONS

What is my reason for wishing to share this type of approach on fish with biologists and ichthyologists? Firstly the understanding of certain information from mythology can sometimes throw light on the physiological cycles of fish - secondly, some ritualized behaviour can be elucidated by a biological approach to the species. While collecting your own data, you surely have gathered information on the behaviours of fish and fishermen that we could share and that could be the beginning of a fruitful collaboration between different disciplines. The comparison of indigenous categories and concepts, traditional wisdom about fish, and traditional management of resources and fishing areas, with the categories of all kinds established by biologists, ichthyologists and other scientists or development experts can provide us with different approaches to the environment and to knowledge. If we could bring together an ethnological approach focusing on an "inside" analysis of the actors' attitudes and the approach of the exact sciences focusing on an "outside" analysis, this should give both sides opportunities for reciprocal enrichment and deeper understanding.

I will end my presentation with a suggestive image: remember that in circumstances of leisure fishing, the fisherman may wildly pursue the fish as prey, only then to let it jump back into the river after having held or stroked it, hence considering the fish as a social partner, almost as a human being.

This paper would not be complete without taking into account the film *The Gift of the Sea* which was presented in the workshop as an illustration of the paper. This 30-minutes colour film is an RFO Noumea production directed by Patrice Sinteff and Elie Peou (Photography Nicolas DOM, underwater photography Claude Michaud, sound Philippe Champenois, editing Serge Parkesian, mixing Franck Richard and Martine Gentis, commentary Patrice Sinteff). It was shown for the first time on November 13th, 1996. Proposed as the contribution of the French University of the Pacific in Nouméa to the 5th Indo-Pacific Fish Conference, the English copy is the work of Pr. Paul de Deckker, Director of the University Centre of New Caledonia, Francine de Deckker, Roy Benyon, Chief



translator of the Pacific Islands Commission and Eric Stimpfing of RFO/ Nouméa. I would like to express my gratitude to all of them here.

The film is about the people of the Chara clan near the village of Tadine on the island of Maré (Loyalty Islands, province on the east side of the main island), who practice traditional fishing for four days that mobilizes some 60 people coinciding with the season of yam harvesting. These contemporary scenes provide a Kanaka version of relations that this group has with the sea and its various inhabitants. The report highlights the exchange of fish and root plants between sea and land clans, and the vital respect of tradition in underwriting reproduction and balance in society.

In the Pacific, each group has special knowledge in various social and religious fields and even if many people might know about it, it stays the property of the group, and only one of its members can speak. Elie Peou from RFO, one of the film-makers and belonging to the clan Chara had agreed to come that evening to answer our questions. I thank him and ask that he express my gratitude to the people of his clan who were kind enough to accept having a film made during this deeply significant ceremony.

**Acknowledgements.** - I deeply thank Professor Roland Billard for having welcomed me in the Laboratory of Ichthyology of the National Museum of Natural History from 1986 to 1993, and Cozette Griffin-Kremer for improving the English translation of the present paper.

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## ON THE BIOLOGICAL INFORMATION CONTENT OF COMMON NAMES: A QUANTITATIVE CASE STUDY OF PHILIPPINE FISHES

by

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**ABSTRACT.** - An analysis was performed on the common names of fishes in the Philippines, based on the COMMON NAMES table of FishBase, a large biological database with key information on fishes. The existence of common names for fish species in 10 Philippine languages was found to depend on the commercial importance, depth range and habitat of these species in the Philippines. Also, the prior hypothesis that certain sounds indicate relative size (i.e., small vs. large) was verified for common names of Philippine fishes ( $P < 0.01$ ). The potential utility of a large and widely available database such as FishBase for storage and analysis of that part of indigenous knowledge that is embedded in common names, and for testing hypotheses that bridge the social and natural sciences, is discussed.

**RÉSUMÉ.** - Sur l'information biologique contenue dans les noms vernaculaires: une étude quantitative sur les poissons des Philippines.

Une analyse des noms vernaculaires des poissons des Philippines a été effectuée à partir de la table des NOMS COMMUNS de Fishbase, qui est une vaste banque de données de noms vernaculaires accompagnés d'informations fondamentales sur les poissons. Ainsi, les noms vernaculaires des poissons dans dix langues des Philippines sont en relation avec leur importance commerciale, leur distribution spatiale et leur habitat. De même, l'hypothèse selon laquelle certains phonèmes indiquent la taille relative des poissons (i.e., petite vs. grande) a été vérifiée pour les noms vernaculaires des poissons des Philippines ( $P < 0,01$ ). On montre l'intérêt potentiel d'une base de données telle que FishBase pour conserver et analyser cette partie de la connaissance autochtone enfouie dans les noms vernaculaires. Cette base de connaissances est également utile pour tester des hypothèses liant sciences naturelles et sciences humaines.

**Key-words.** - Fish, Philippines, Common names, Database, Linguistics, Sound symbolism.

When Linnaeus established his binomial system for naming biological species, he claimed that all generic names « should be apt in meaning, easy to say and remember, and pleasant to hear » (Stern, 1959). The review of Berlin (1992), on the other hand, suggests that people who have close links with their natural habitat follow, when naming species, a system of nomenclature « that can be best explained in terms of human beings' similar perceptual and largely unconscious appreciation of the natural affinities among groupings of plants and animals in their environments » (p. xi). Further, from the body

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The present paper is ICLARM Contribution n° 1422.

of this work, organized « in the spirit of hypotheses to be tested » (Berlin (1992, p. xiii), the following attributes can be identified as making organisms likely to be named: 1) commonness; 2) striking appearance; 3) ease of observation; 4) large size relative to humans. The last of these attributes also affects the phonetic value of the names that are given (Berlin 1992, p. 234 and following, and see below).

A test of (1) may be performed given information on the relative abundance of a species within its local habitat. Hypotheses related to the second of these attributes can be tested whenever a taxonomy is complete, as species of striking appearance are often the only members of monotypic families. Thus, a test of (2) may involve plotting the frequency of occurrence of common names of species in monotypic families vs. their frequency in similar species. Testing (3) can be done, in fishes, using information on the vertical distribution of a species within the water column. Testing attribute (4) requires standardized measures of size, e.g., the maximum length reported from a given species of fish.

Berlin (1992, p. 234 ff) also analyzed size-sound symbolism in common names of birds named by the Huambisa people of the Peruvian rain forest. The results of his study suggested a preponderance of high frequency sounds (i.e., syllables with "i") in the Huambisa common names for small birds, and conversely for low frequency sounds ("a") in large birds. Berlin (1992, p. 247 ff) suggested that this sound-size symbolism also applied to animals which do not (usually) emit sounds audible to human ears (e.g., to fishes), and that it is widespread through the world's indigenous languages.

In this study, we test hypotheses related to the four above attributes, using over 1200 common names of fish in 10 Austronesian languages of the Philippines (Ruhlen, 1991), after accounting for borrowings from Indo-European languages (i.e., Spanish and English).

## MATERIALS AND METHODS

Over 1,200 fish common names used in 10 Philippine languages ("local names") were obtained from the Common Names Table of FishBase (Palomares and Pauly, 1997), an electronic encyclopedia on key information on fish (Froese and Pauly, 1997). Associated information on monotypy, maximum size, depth range and habitats were obtained from the Species Table (Froese *et al.*, 1997). Information on the commercial importance of various species was obtained from the Countries Table of FishBase (Froese and Garilao, 1997).

The origin of these local names was determined from dictionaries (Kaufmann, n.d.; McKaughan and Macaraya, 1967; Panganiban, 1972; Wolff, 1972; Santos, 1978; Makabenta, 1979; English, 1986; Williams, 1987; Geladé, 1993; Hassan *et al.*, 1994). To exclude names that were brought to the Philippines during the 300 years of Spanish occupation, all names were matched against the Spanish common names in FishBase, and attributions verified by the authors. English names were similarly deleted. Names whose origin could not be determined were excluded from the analyses.

Over 600 unique, non-composite local names were identified, in 10 languages. These names were arranged in ascending order of the maximum length reported for each fish species, then grouped in tiers, i.e., total sample/3. The first and last thirds were identified as "small" and "large" fish, respectively. This approach differs from that used in Berlin (1992, p. 248) who defines small fish as those below a fixed cutoff length of 10

inches, and above for large fish. Once the size groups had been established, first syllabic vowels were counted and recorded for each size group, as were first syllabic occurrences of "ti" and "ta", identified as typical high and low frequency sounds, respectively.

## RESULTS AND DISCUSSION

Table I lists the Philippine languages from which 1286 names were obtained, by language (family) of origin. Table II summarizes the data for our test of Berlin's first attribute. As might be seen, a large proportion of fish used in artisanal fisheries (41%) in the Philippines do not have local names assigned to them whereas only 10% of commercially very important fish species lack such names. From this, we conclude that indeed, common species, supporting large fisheries, tend to be named more often than the scarcer species contributing to the multispecies artisanal catches.

Table I. - Origin of fish names in 10 Philippine languages. Data from FishBase (Palomares and Pauly, 1997); the names in Kapampangan and Mapun were not used in tests of sound-size hypotheses.

Language	Total	Austronesian origin	Spanish or other Indo-European origin	Undetermined origin
Tagalog	336	283	39	14
Cebuano	335	322	4	9
Bicolano	216	204	9	3
Maranao/Sama/Tausug	115	113	1	1
Kuyunon/Tagbanwa	83	73	6	4
Ilokano	62	56	3	3
Banton	59	53	2	4
Pangasinan	43	38	4	1
Kapampangan	35	31	4	0
Mapun	2	2	0	0

Table II. - Distribution of 2218 named and unnamed fish species and of 74 monotypic species according to their importance in Philippine fisheries. Data from FishBase (Froese and Pauly, 1997). "Importance" is a multiple choice field in FishBase; local names included from Tagalog (41% of all local names), Cebuano (25), Bicolano (8), Maranao/Sama/Tausug (6), Kapampangan (6), Kuyunon/Tagbanwa (3), Ilokano (2), Banton (2) and Pangasinan (2). The remaining 5% are English names used in the Philippines.

Importance	All Philippine species	Species with local names (%)	Monotypic species	Monotypic spp. with local names (%)
Highly commercial	20	90.0	2	50.0
Commercial	493	88.6	25	88.0
Minor commercial	209	74.0	11	82.0
Artisanal fisheries	1496	41.0	36	22.0

Concerning the second of Berlin's attributes, pertaining to striking characters in named species, we found that 74 monotypic species occur in the Philippines, of which 40 have local names assigned to them in the various Philippine languages. Overall, a smaller percentage of monotypic species has names than would be expected given their role in the fisheries (Table II). Thus, we can not confirm Berlin's attribute (2), at least not in the form we have tested it.

FishBase presently lists 936 shallow water (0-99 m) species occurring in the Philippines. Of these, 55% have local names. Of the 105 deep water (100 m and deeper) species, only 24% have local names. Generally, species inhabiting bathydemersal, bathypelagic and benthopelagic habitats lack local names, which confirms Berlin's third attribute (Table III).

Table IV shows that large fish tend to be named more frequently than small fish, thus confirming Berlin's 4<sup>th</sup> attribute.

The distribution, in Philippine languages, of first syllabic "a" and "i" was not significantly related to fish size (data not shown). On the other hand, small and large fishes differ significantly, in Philippine languages, in terms of the occurrence of first syllabic "ti" and "ta" (Table V).

This confirms Ohola (1984) who hypothesized that « words denoting or connoting SMALL or SMALLNESS [...] tend to exhibit a disproportionate incidence of vowels and/or consonants characterized by high acoustic frequency [while] words denoting LARGE use segments with low acoustic frequency ». The results in table V also extend to the Austronesian language family the validity of a sound-meaning hypothesis (Berlin, 1992) previously verified for languages of the unrelated Amerindian family (Ruhlen, 1991).

Habitat	Number of species	Spp. with local names (%)
Reef-associated	1164	56.9
Pelagic	175	70.3
Demersal	673	51.4
Bathypelagic	17	5.9
Bathydemersal	39	33.3
Benthopelagic	134	28.4

Table III. - Habitat of named and unnamed species in the Philippines. Data from FishBase (Froese and Pauly, 1997). "Habitat" is a multiple choice field in FishBase.

Size group (cm)	Species named (%)
Small (1-13)	286 (40.8)
Medium (13-31)	415 (59.2)
Large (31-1370)	476 (67.9)

Table IV. - Philippine species ( $n = 2103$ , in tiers of 701 spp. each) with local names, by maximum length. Data from FishBase (Froese and Pauly, 1997).

Size group (cm)	Count of 'ti'	Count of 'ta'
Small (< 30)	8	17
Large (> 100)	0	21

Table V. - First syllabic use of the letters "ti" and "ta" in the first and last thirds of 688 local names of fish in Philippine languages (from FishBase; Froese and Pauly, 1997). The size-specific counts of "ti" and "ta" are significantly different (Fisher's exact test of independence;  $P < 0.01$ ).

The results presented here indicate that a large database in which local names are linked with scientific nomenclature and biological attributes (such as FishBase) can be used to great advantage to test, in quantitative terms, hypotheses relevant to linguistics and/or cultural anthropology. This is perhaps surprising given the very different purposes for which FishBase was originally developed, and given the widely held perception among social scientists that culture- or language- related hypotheses are not amenable to quantitative testing. On the other hand, our results are consistent with the suggestion of Wilson (1998) that, because of evolutionary constraints, research on humans and the world they live in should lead to results that show consilience, i.e., that "jump together".

**Acknowledgements.** - We wish to thank Drs. Rainer Froese and Roger Pullin for their useful comments and advice and Ms. Alice Laborte for helping with the database queries.

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ACHEVÉ D'IMPRIMER  
EN JUILLET 1999  
SUR LES PRESSES  
DE  
L'IMPRIMERIE F. PAILLART  
À ABBEVILLE

DÉPÔT LÉGAL : 3<sup>e</sup> TRIMESTRE 1999  
N<sup>o</sup> D'IMP. 10731

